GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 3/6/79

Project Title: Identification of Major and Minor Classes of Natural Organic Substances Found in Drinking Water

Project No: E-20-657 (Prior No. A-1983) Green card

Project Director: Dr. S. C. Havlicek

Sponsor: Environmental Protection Agency

Agreement Period: From 1/15/79 Until ~~6/15/79~~ 9/5/79

Type Agreement: Contract No. 68-01-4480

Amount: $37,795.59

Reports Required: Monthly Progress Reports, Graph Financial Management Reports; Final Report

Sponsor Contact Person (s):

Technical Matters

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EPA, Office of Water Supply
Criteria & Standards Division
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Washington, D. C. 20460

Contractual Matters

(thru OCA)
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Negotiated Procurement Section
Crystal Mall #2, Rm. 701
(PM-214-C)
Washington, D. C. 20460

Defense Priority Rating: n/a

Assigned to: Civil Engineering (School/Laboratory)

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CA–3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 2/22/80

Project Title: Identification of Major and Minor Classes of Natural Organic Substances Found in Drinking Water

Project No: E-20-657

Project Director: Dr. Havlicek

Sponsor: Environmental Protection Agency

Effective Termination Date: 9/5/79

Clearance of Accounting Charges: 9/5/79

Grant/Contract Closeout Actions Remaining:

- x Final Invoice and Closing Documents
- _ Final Fiscal Report
- x Final Report of Inventions
- x Govt. Property Inventory & Related Certificate
- _ Classified Material Certificate
- _ Other _____

Assigned to: Civil Engineering (School/~~Laboratory~~)

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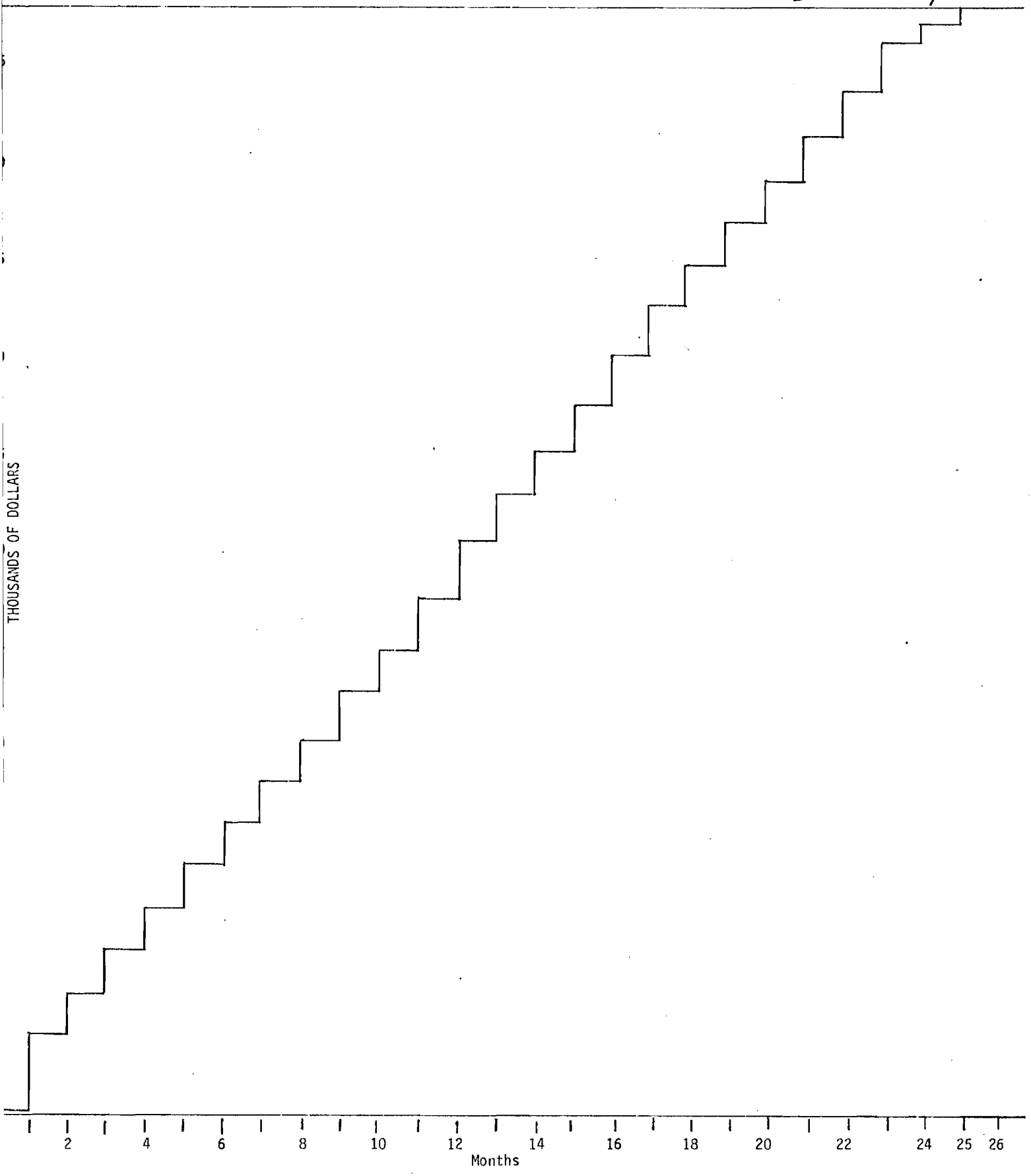
CA–4 (1/79)

A-1983
Annual Report
E-20-657

FINANCIAL MANAGEMENT REPORT

GEORTIA TECH RESEARCH INSTITUTE - CONTRACT No. 68-01-4480

$236.649



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JULY

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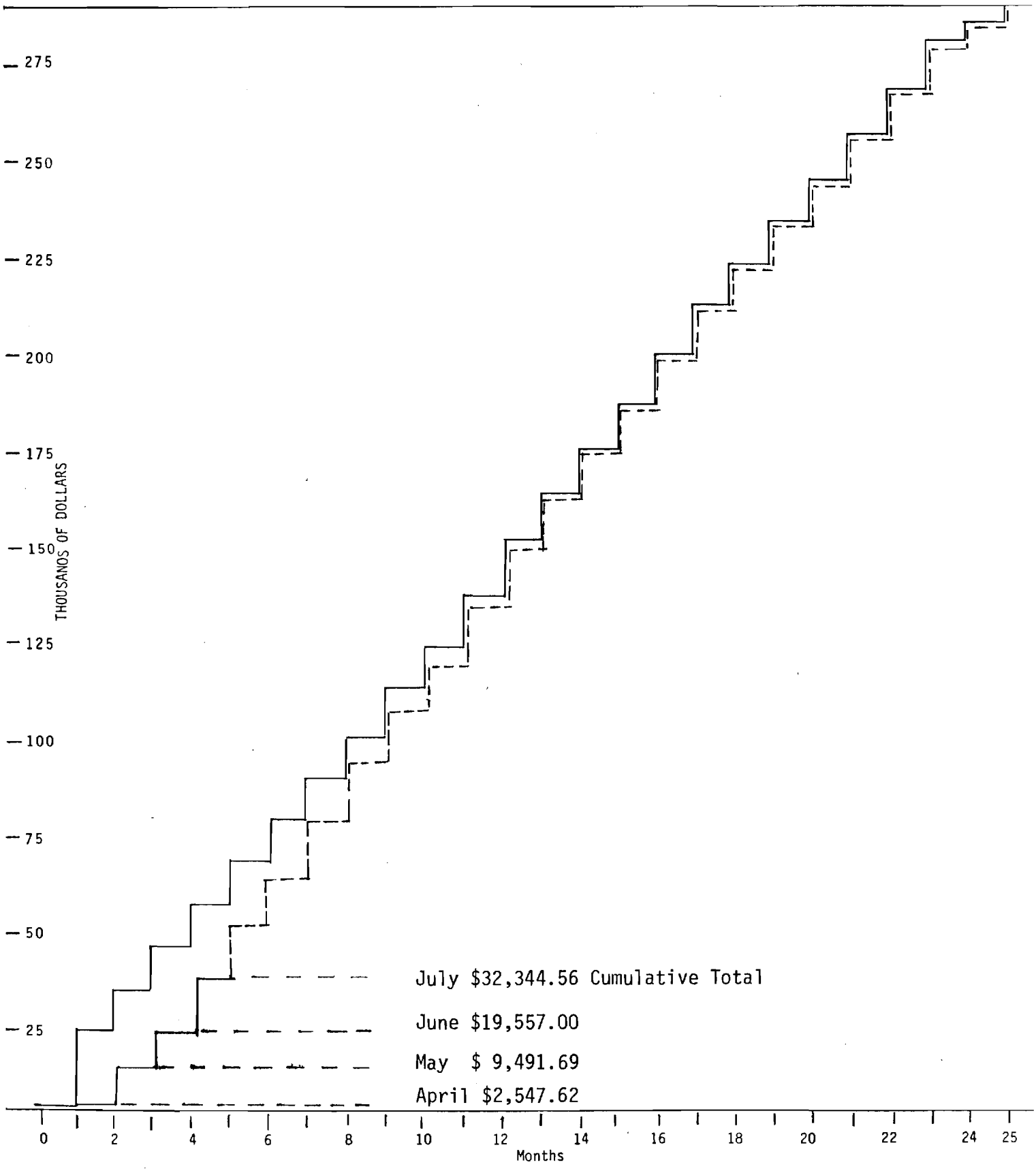

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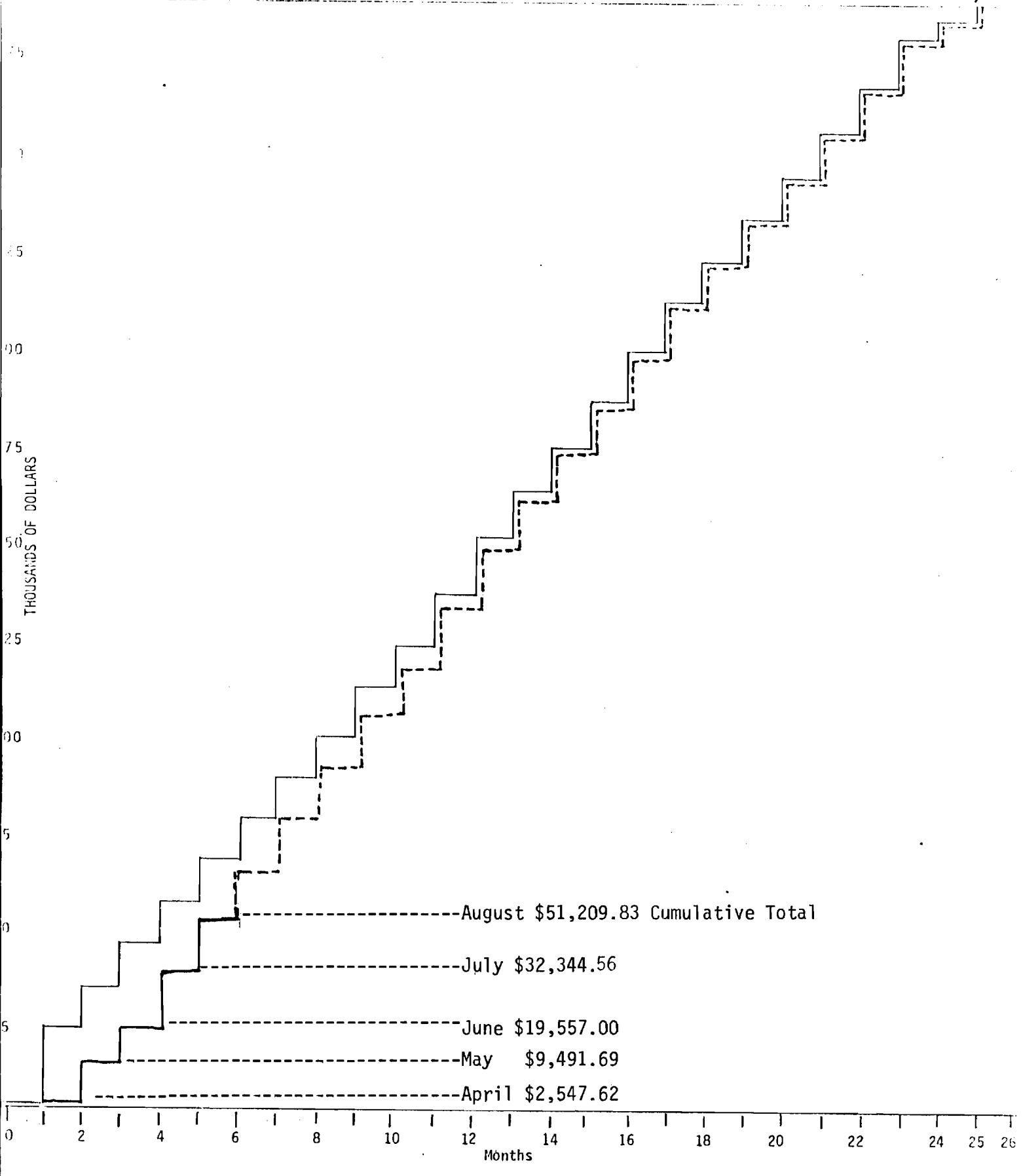

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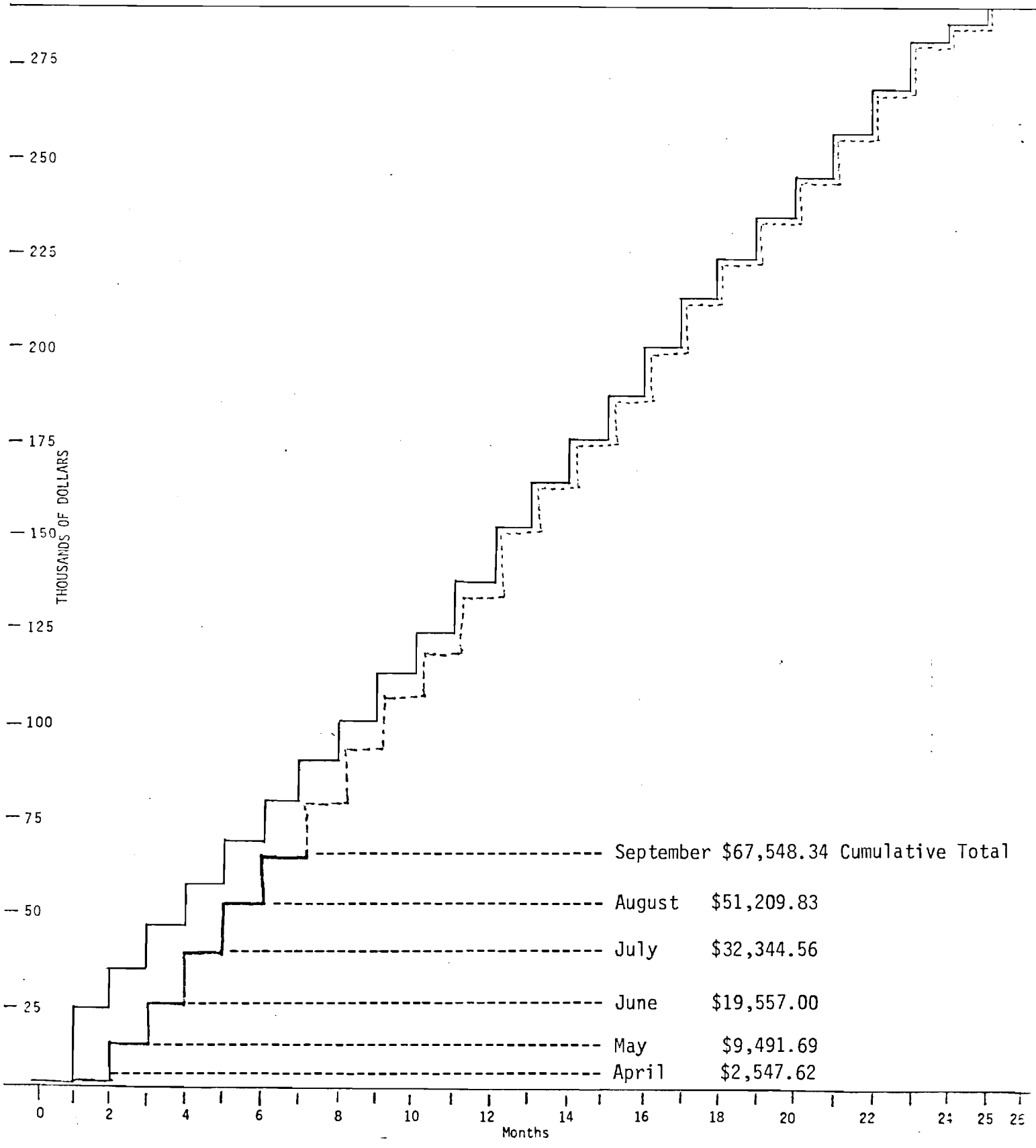

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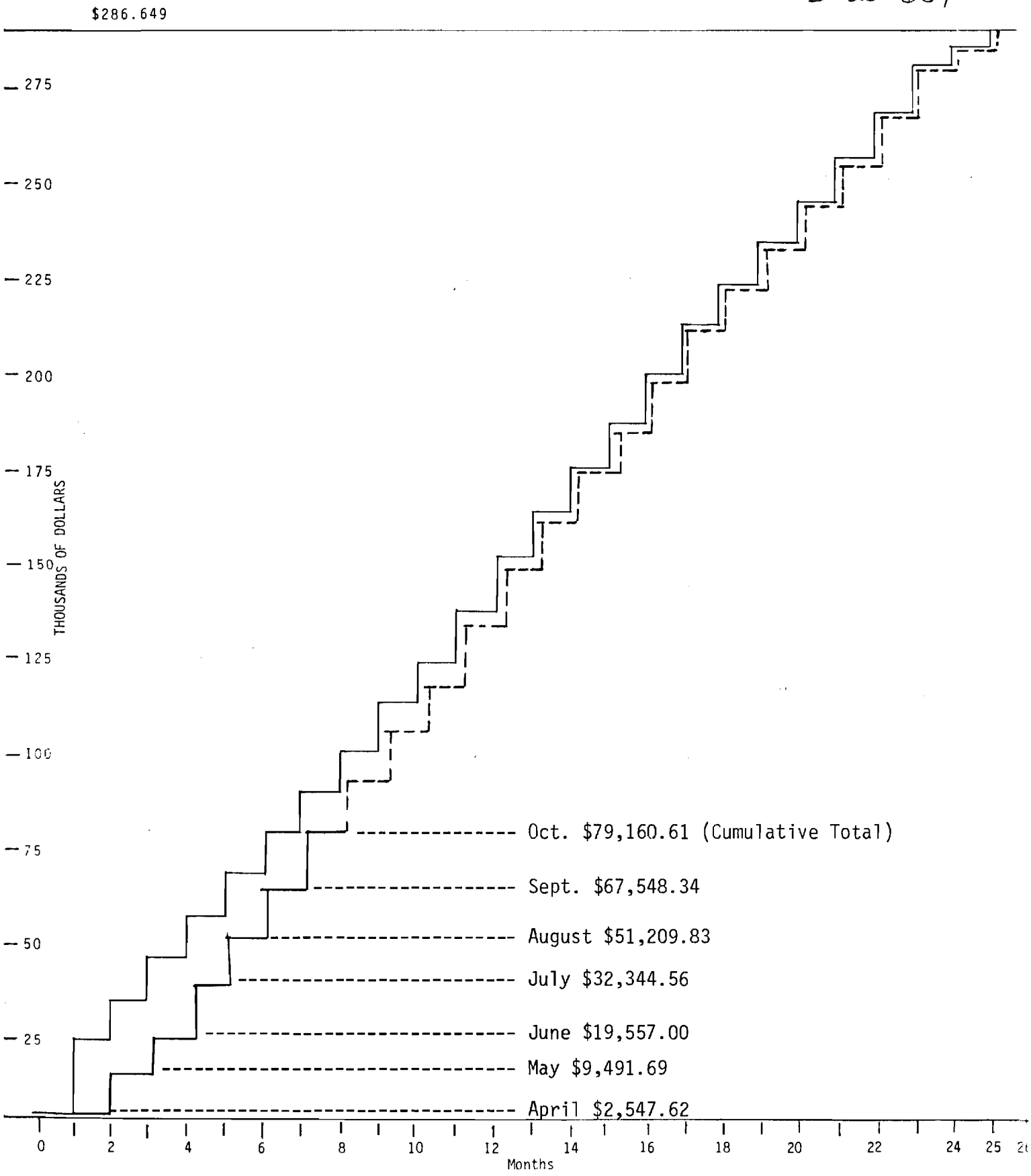

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NOVEMBER

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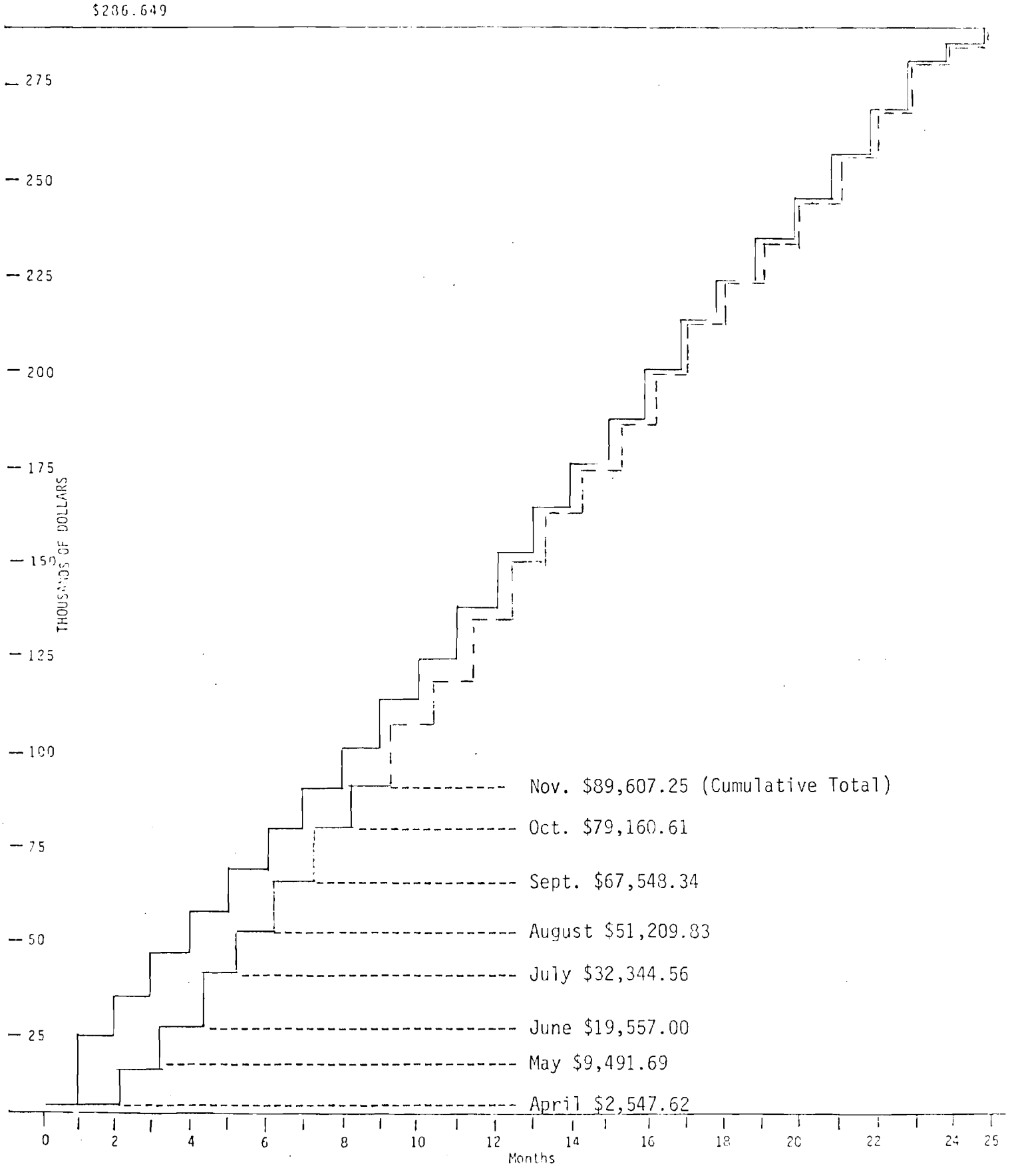

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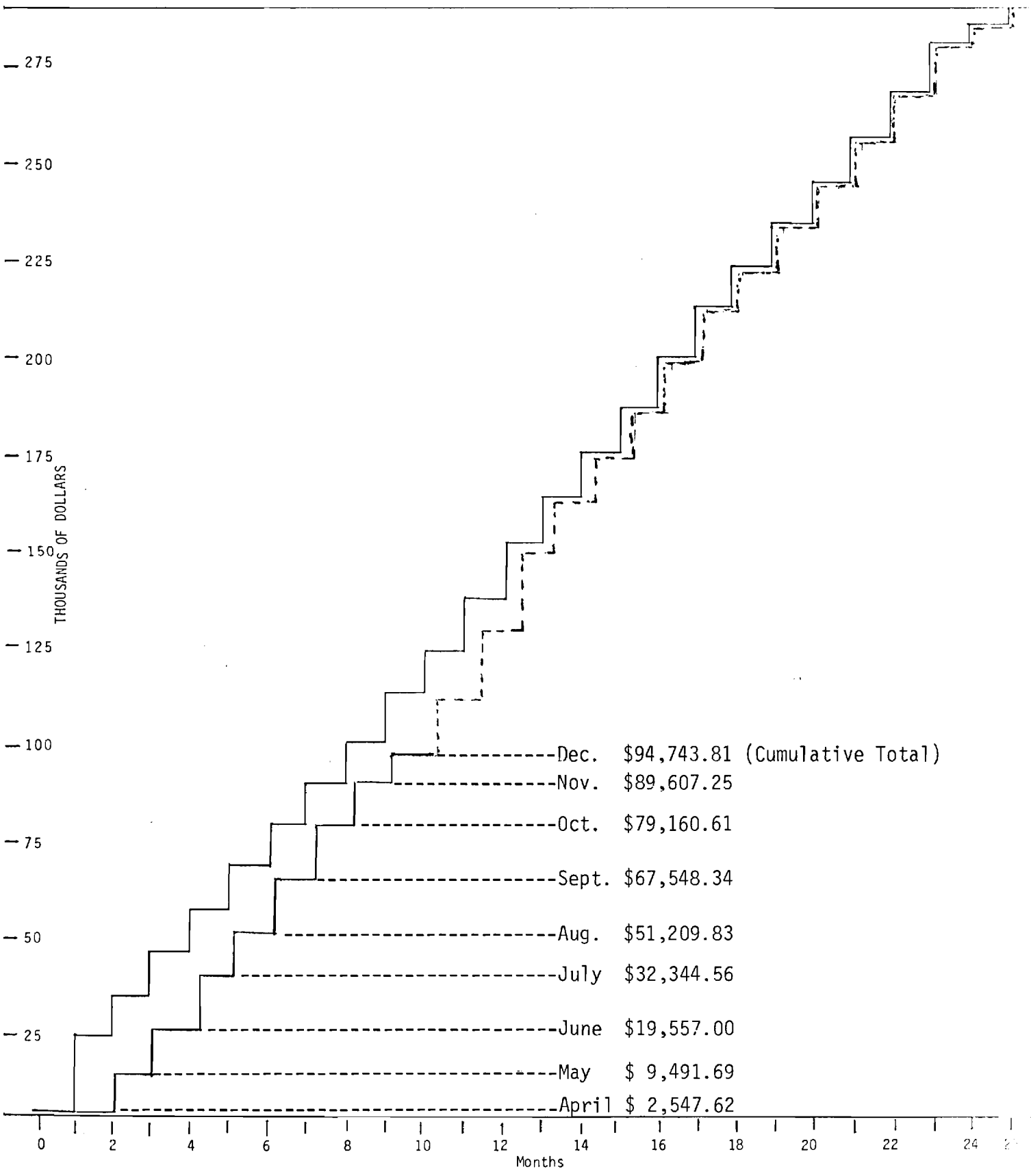

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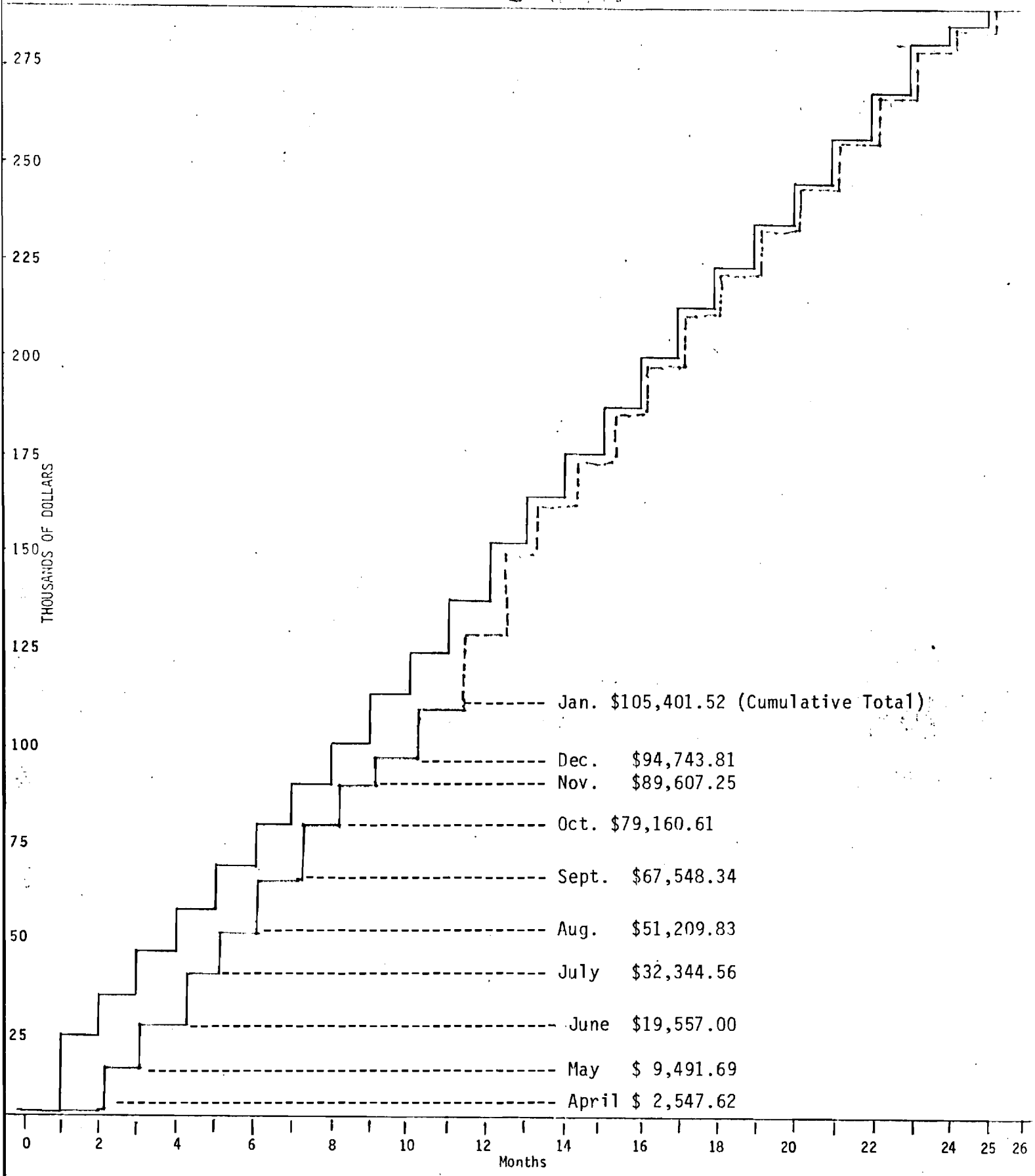
E-20-657



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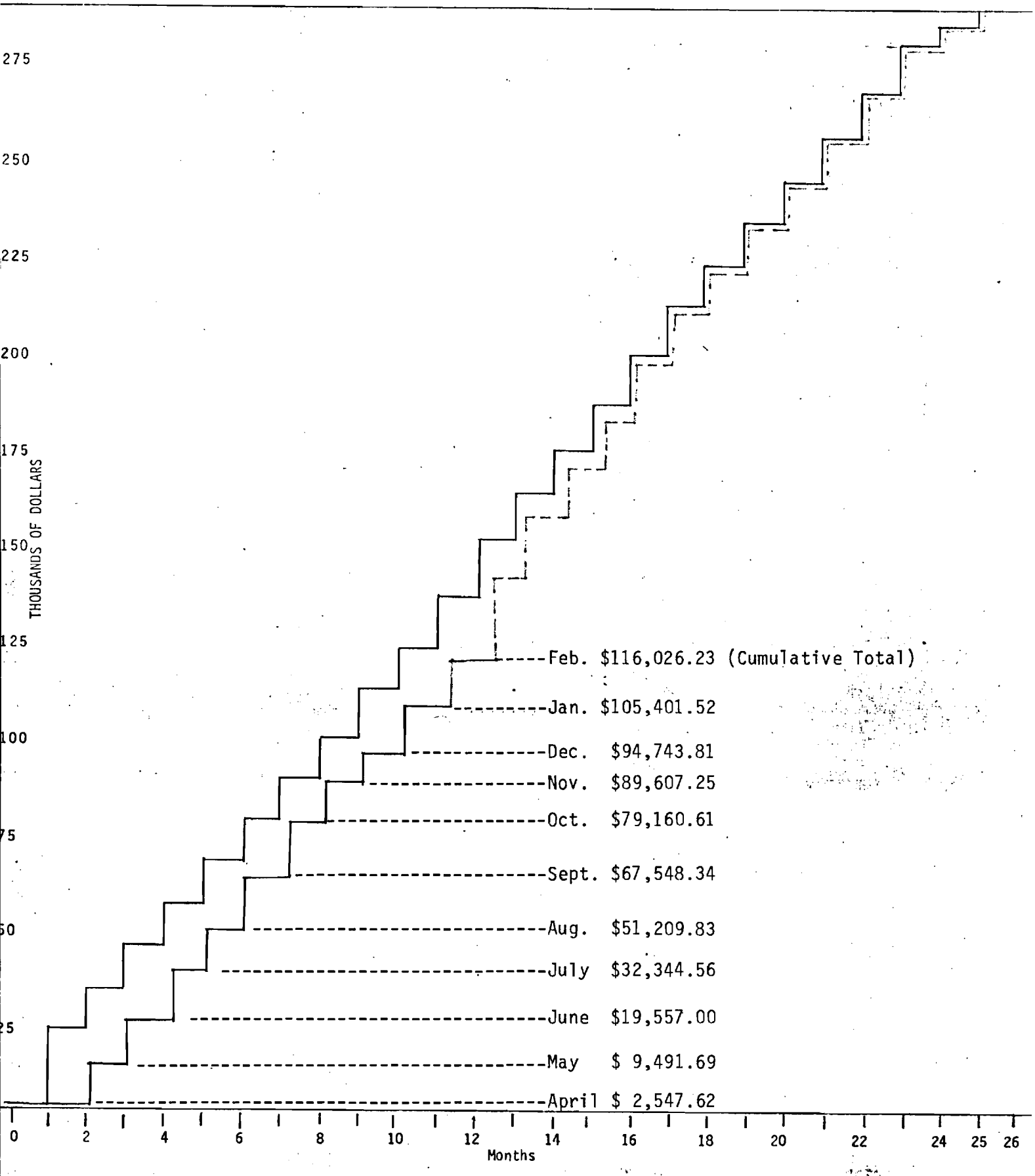

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E-20-657

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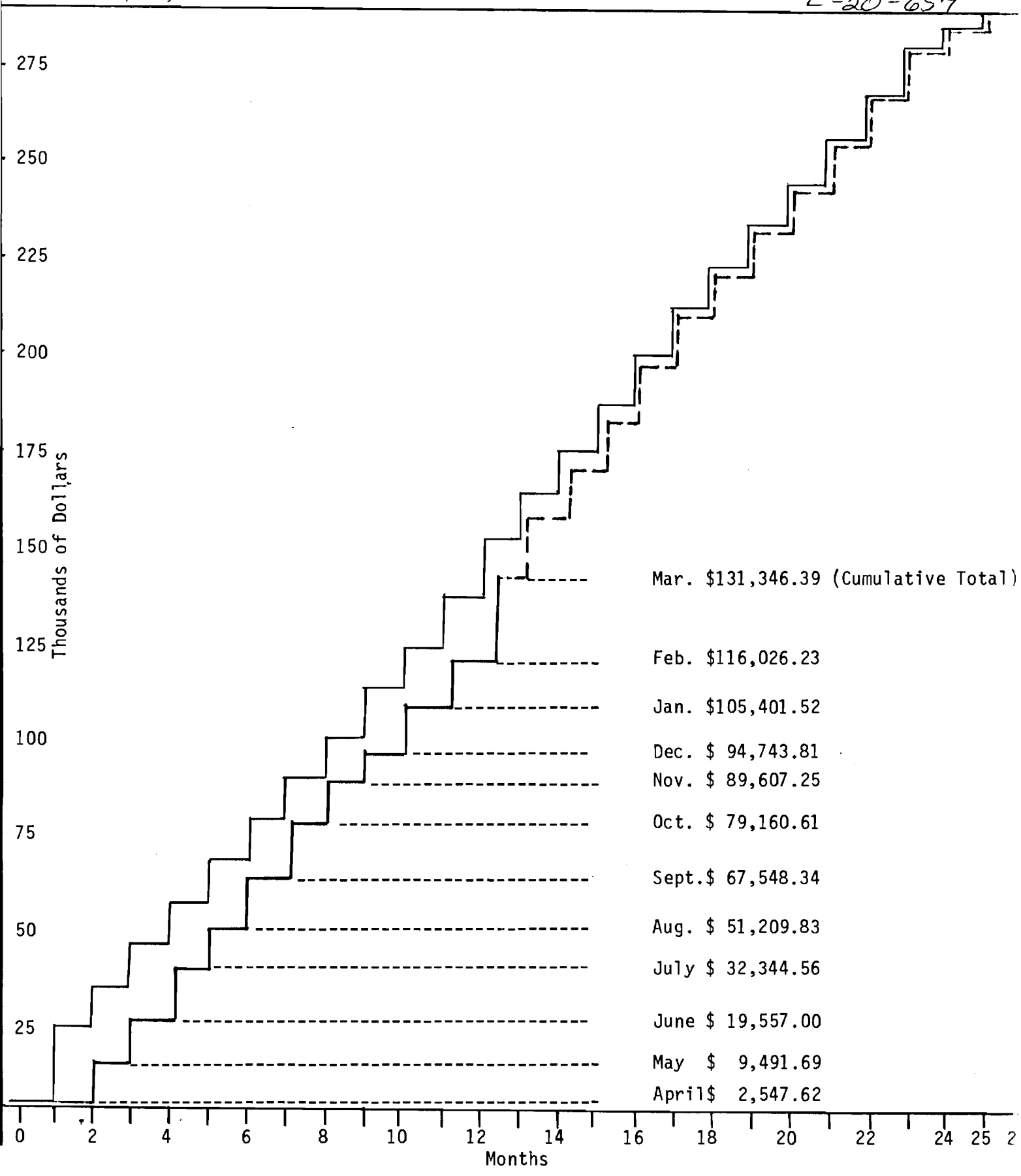

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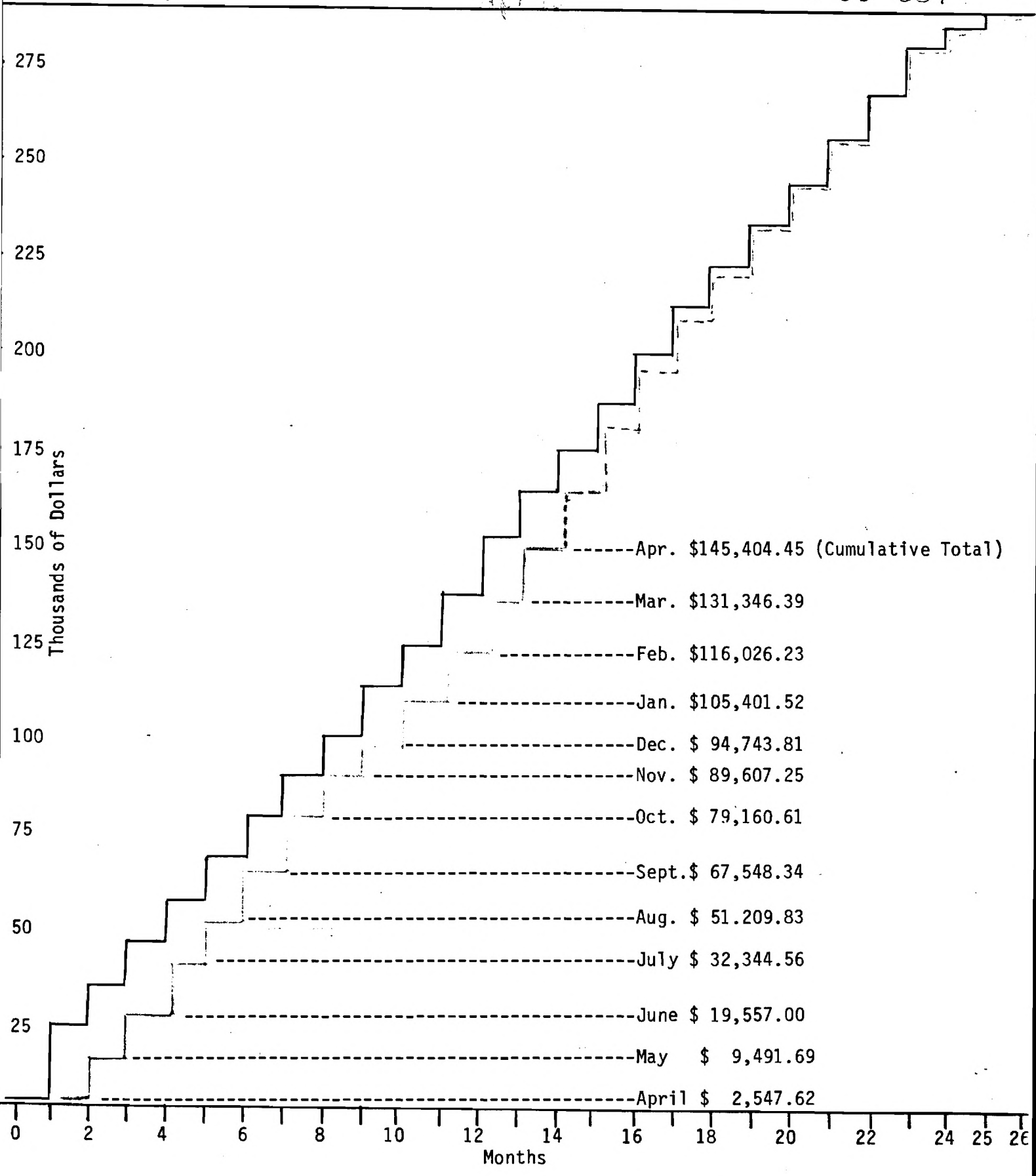

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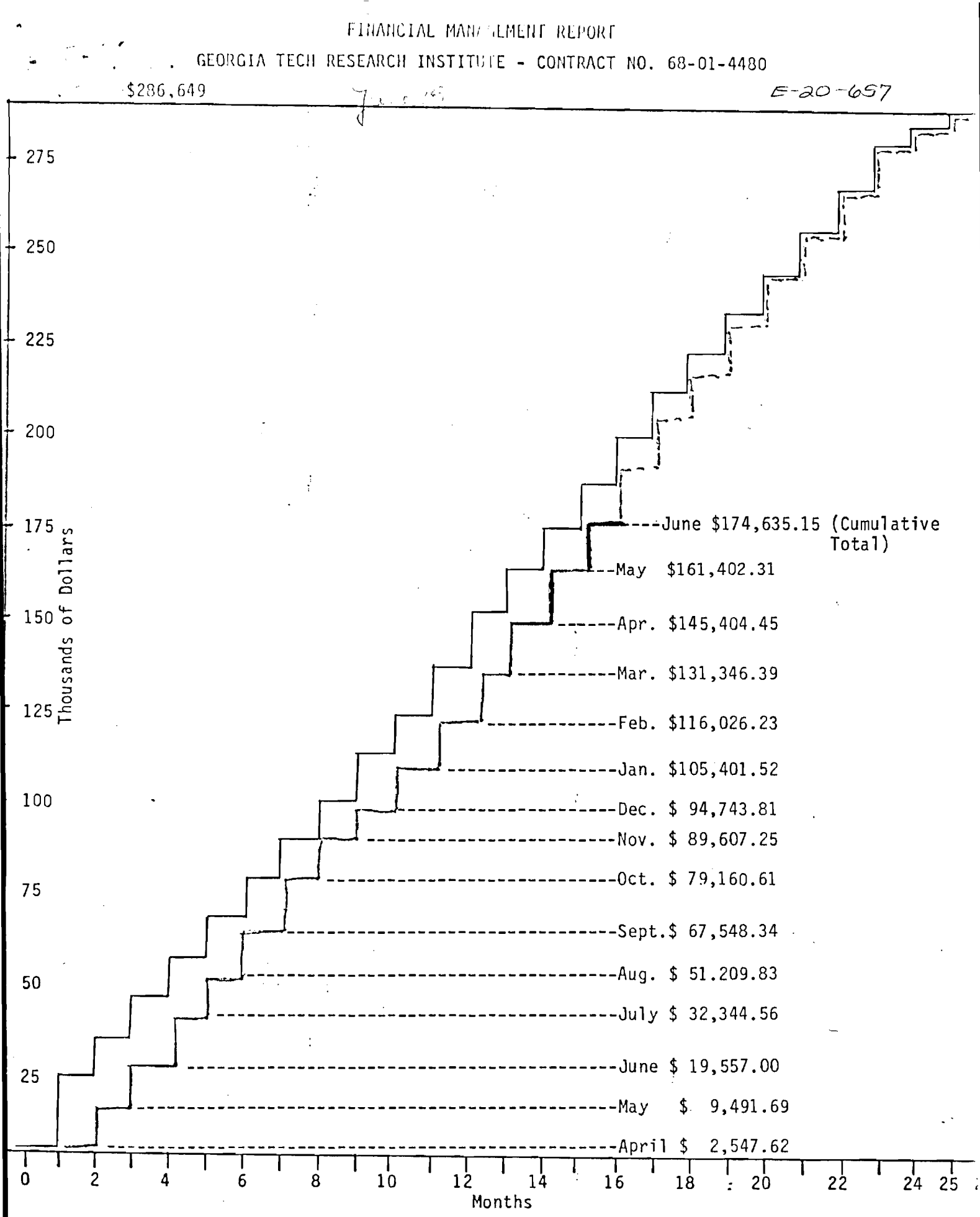

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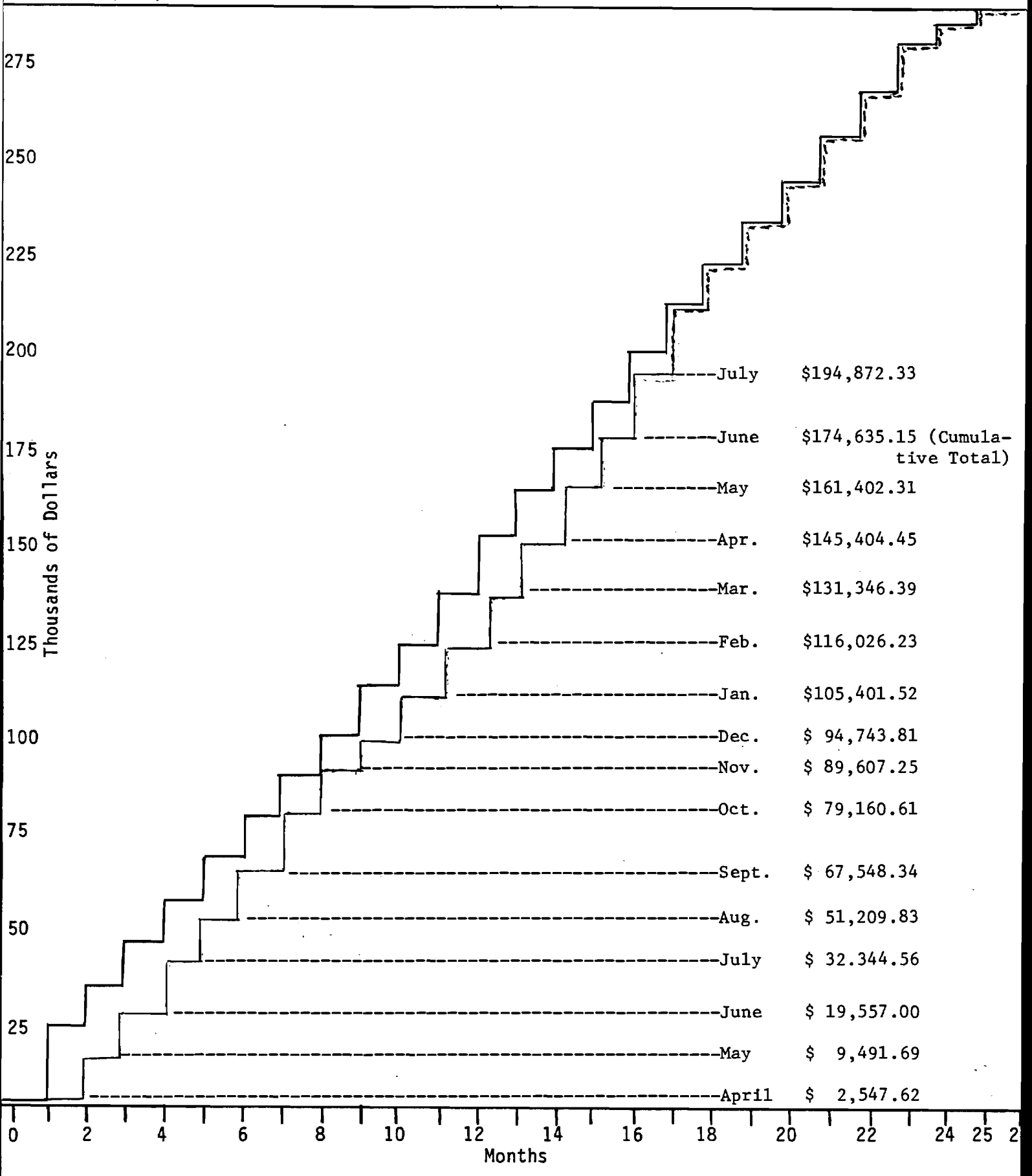
GEORGIA TECH RESEARCH INSTITUTE - CONTRACT NO. 68-01-4480

E-20-657

$286,649 JULY 1978



E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURUAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

May 12, 1977

by

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68-01-4480

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DISCLAIMER

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources. Considerable progress has been made in characterizing this material. The advances made to date suggest a more open chain, aliphatic structure than has been suggested by the literature. A new technique for measuring the carboxylate acidity has been developed and employed for the characterization of humic substances. Oxidative degradations have been carried out under a wide variety of conditions using methylated aquatic humic substances. Extremely mild conditions have brought about vast improvements in the yields. The powerful techniques of capillary GC/MS analysis have successfully separated these highly complex reaction mixtures into hundreds of individual components, some of which have already been identified and compared with authentic samples.

Several model compounds have been reacted with disinfectants under a variety of conditions. Of these, hesperetin has been used more extensively than most of the others - chiefly with chlorine. During this reporting period, some preliminary results have been obtained using the methods of ion-pair liquid chromatography which suggest there may be a higher degree of chemical comparison between what happens to hesperetin and what happens to aquatic humic matter when the two materials are subjected to disinfection with chlorine.

A mini-pilot water treatment facility has been constructed so that model compounds and classes of natural isolates can be subjected to disinfection under realistic conditions. This facility has been running successfully for several months and is now being modified so that the granular activated carbon filter can be fluidized by backflushing. Samples of carbon are now on hand and work is expected to begin shortly.

During this reporting period work was initiated involving the treatment of aquatic humic substances with bromine in an effort to spectroscopically define the changes in chemical bond type occurring during the reaction. In addition to noting the formation of new C-Br bonds, a possible diminution of some C=O bonds and a lack of change in the C-O region, a crystalline product mixture was obtained which will be resolved into its components in the near future.

Workup, separation and isolation of the product mixture resulting from the treatment of aquatic humics with iodine and sodium hydroxide have resulted in the identification of iodoform, methylene iodide and a number of other products. In view of the positive results regarding the uptake of iodine, further work under milder conditions is contemplated.

Characterization of the product mixture resulting from the treatment of aquatic humic materials with chlorine in the mini-pilot facility has resulted in the isolation of a series of compounds containing one isoprene unit. Many of these are chlorinated compounds which have not been reported previously. Thus their discovery represents a fundmental advancement of our knowledge of the reactions taking place during the normal disinfection of drinking water. Work in this area will be given top priority during the next reporting period.

In general, the project is on schedule, albeit with an increasing emphasis on the treatment of aquatic humics with disinfecting agents and a decreasing emphasis on model compounds. All major instrumentation is running smoothly and no problems are foreseen.

I. PERSONNEL

No changes in personnel have taken place during the reporting period.

II. EQUIPMENT

A new flame ionization detector is being installed on the Finnigan model 9500 GC (not the one attached to the mass spectrometer) so that a universal detector will be available on an instrument which can be used with capillary columns. A modified Lupton detector is being constructed for use with this instrument. The new design, which is approaching the working model stage should more readily accommodate capillary columns and will therefore have direct utility in carrying out this project more effectively. While neither of these specific activities is being supported by program funds, they have been discussed because the completed tasks will enable us to more effectively carry out the work of characterizing the reactant and product mixtures encountered in connection with this project.

A gas-phase ultraviolet ozone detection apparatus has been assembled and is about to be used with the various types of ozone generators in an effort to better characterize the outputs in terms of O_3 and oxidizing power. A Miran infrared detector will also be used in this experiment so that we will have two independent methods for determining ozone concentration. Any failure to correlate chemical oxidizing capacity with O_3 levels would be strong supportive evidence that other oxidants are present. A sketch of the apparatus is shown in Figure 1.

It is envisioned that the output from the Welsbach, corona, long-wavelength UV and short-wavelength UV generators will be characterized using this apparatus. It should be pointed out that a strong air flow will be directed across the path of the light from the mercury lamp so that it will not have the opportunity to build up significant ozone levels by virtue of the interaction of the UV light with atmospheric oxygen.

The GC/MS and other systems continue to operate smoothly as they have for the last several months.

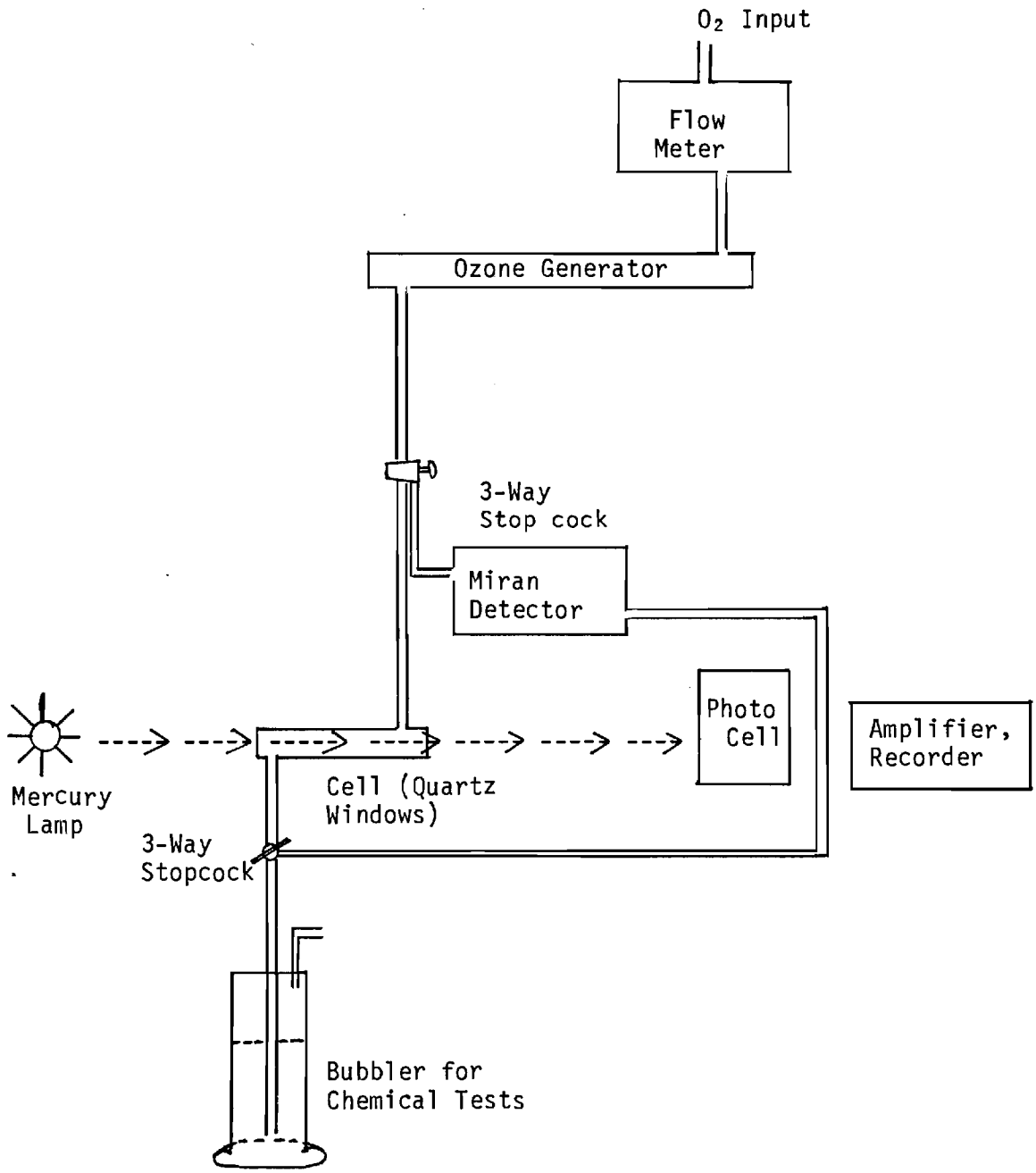


Figure 1. Apparatus for Characterizing O_3 Output

A new software package is being substituted for the one presently in place. Hopefully this will help resolve minor problems resulting from occasional mis-assignment of m/e valves and will therefore help us clear our backlogged samples.

III. ESTIMATION OF CARBOXYL GROUPS IN AQUATIC HUMICS

The new approach outlined in last month's progress report (Section IV., p 2) has been perfected. It will be recalled that the traditional approach involves the displacement of acetic acid from a 0.1 N calcium acetate solution followed by titration of the liberated acid with sodium hydroxide. The inflection marking the endpoint is often indistinct (dashed line, Figure 2) presumably due to the interferences caused by the passage of polymeric materials through the filter paper which is used to separate the calcium humate from the acetic acid just prior to titration. In the new method the filter paper is replaced by an Amicon UM 2 membrane which is designed to reject all compounds with a molecular weight greater than 1000. When this step is carried out (under nitrogen), the filtrate is colorless and titration with standard base gives a sharp inflection (solid line, Figure 2). The results obtained by this method are slightly lower than those obtained by the old method due to the fact that the endpoint, in addition to being more clearly defined, requires a slightly lesser volume of base. This finding would indicate that published methods may be overestimating the carboxylate functions by about 1.5 meq/g.

IV. REACTION OF AQUATIC HUMICS WITH BROMINE IN ACETIC ACID

Aquatic humic material (sample M/30, 100 mg) was suspended in acetic acid (4 ml) to which bromine (0.3 ml) was subsequently added dropwise with swirling. The reaction mixture was then heated (60^{o}) for 10 minutes during which time the suspension gradually dissolved. The solvent and unreacted bromine were removed overnight using a slow nitrogen sweep. The resulting deep red flakes were further dried and then examined using the infrared spectrophotometer. A new band appeared in the product at 485-635 cm^{-1} which can be attributed to C-Br stretching. The relative intensity of the 1600-1650 cm^{-1} region is decreased somewhat, possibly due to the bromination of $COCH_2CO$ functions. The products are presently being subjected to further workup and analysis.

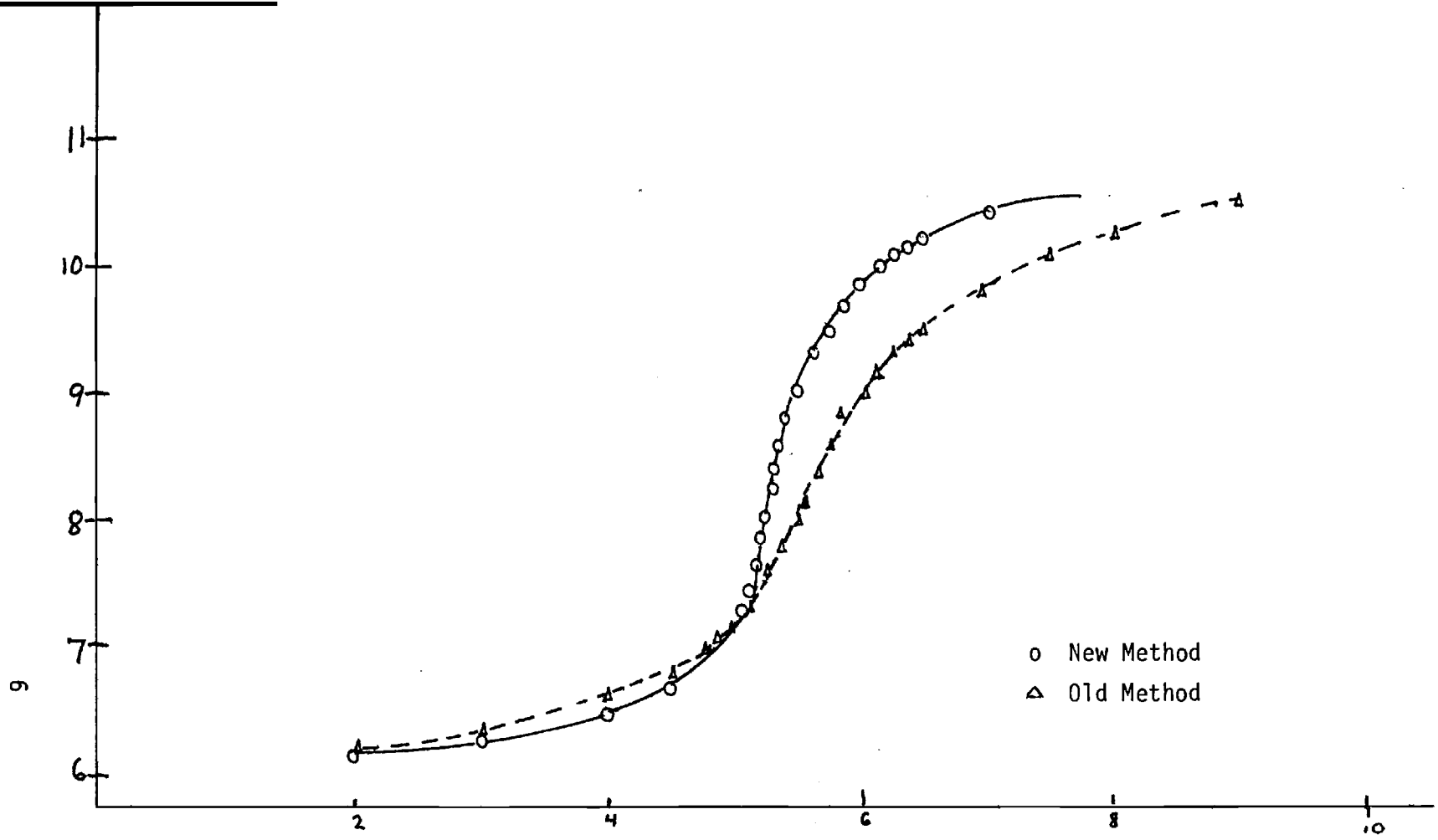


Figure 2. Improved Method Estimation of Carboxylate Groups (normalized data).

V. REACTION OF AQUATIC HUMICS WITH IODINE IN SODIUM HYDROXIDE SOLUTION

It will be recalled that the reaction of 500 mg of aquatic humic material with aqueous iodine and sodium hydroxide was described in one of our earlier reports (Section VII, p. 15, Feb. 6, 1978). Direct ether extraction provided iodoform. Acidification, filtration and extraction with both ether and ethyl acetate provided additional organic material which has now been examined by GC/MS. An analysis of these data indicates that additional iodinated compounds are present. At present only the EI spectra are available. No attempt has been made to derivatize acidic and phenolic groups. Since this was only a qualitative experiment, the rigid exclusion of possible solvent contaminants was not practiced. Therefore, the presence of such chemical entities as benzene, pyridine and toluene in the isolates could well be due to contamination while the acetates and ethyl esters might possibly be artifacts brought about by exchange reactions with the ethyl acetate used as an extractant. The iodinated compounds, however, appear to be real and worthy of note. Of these, methylene iodide is perhaps the most interesting (see Figure 3). The results are summarized in Table I. Representative spectra are presented following the table. The total ion chromatogram is presented in Figure 4.

In closing this section it would be appropriate to note that since the presence of iodine is indicated in other chromatographic peaks and since iodoform and methylene iodide have been identified in the product mixture, it is therefore evident that reactions are taking place which may have significance beyond the structural elucidation of aquatic humic substances. It is the writers' opinion that further work in this area would be useful. We would propose to rerun the original oxidation and/or conduct a run on the mini-pilot facility using conditions which would be more appropriate for disinfection.

VI. JAR TESTS FOR THE FLOCCULATION OF AQUATIC HUMIC MATERIAL

A second series of jar tests was made to develop coniditons which would enhance flocculation during treatment of aquatic humic materials with disinfecting agents. The monthly report for March, 1978 described a series of jar tests run using pH control (buffers) reagents as part of the mixture tested. These tests showed that high dosages of ferric chloride and aluminum sulfate were required to produce flocculation. A second series of jar tests was run

SAMPLE: HUMICS OXIDIZED WITH NAOH

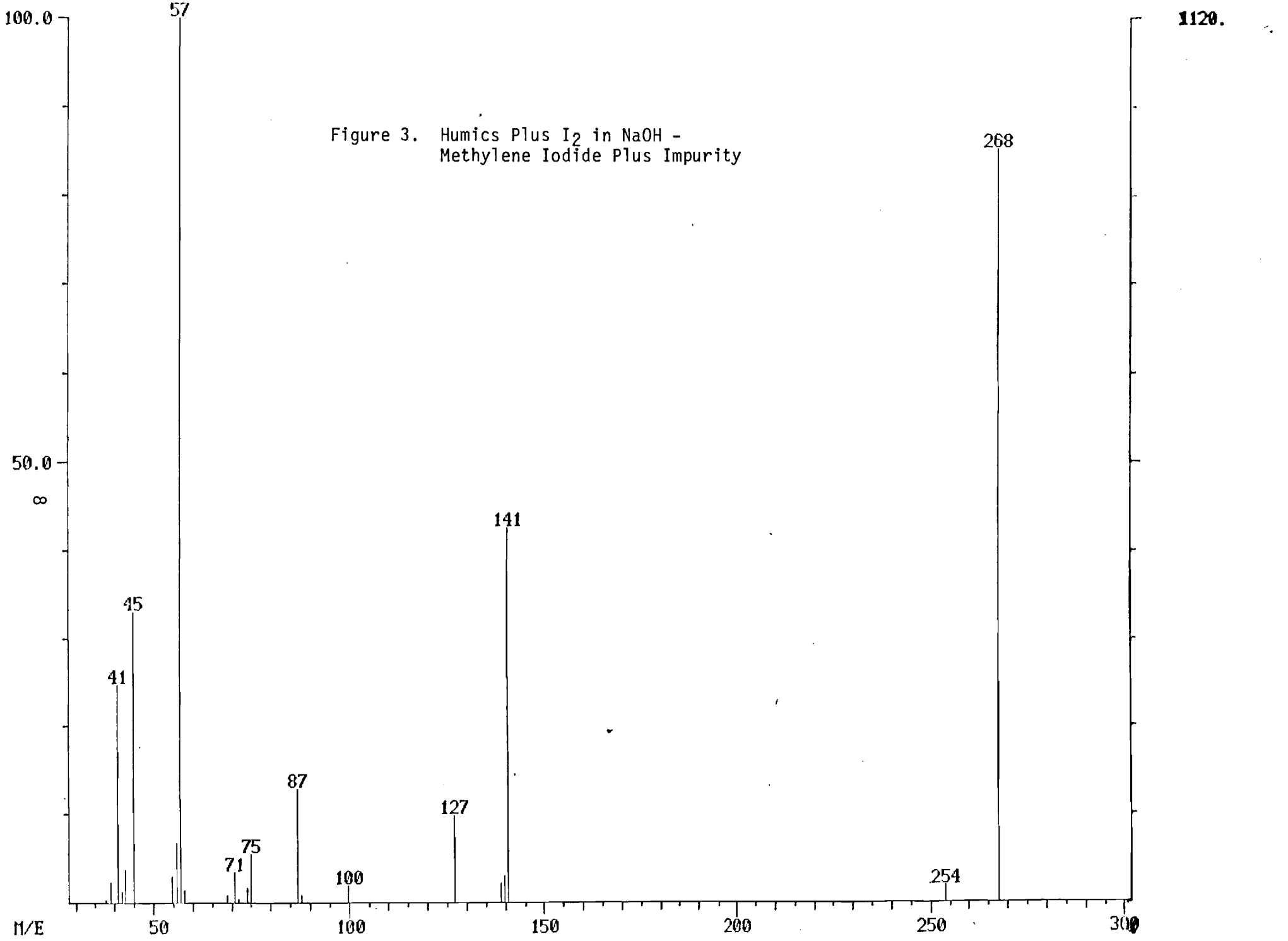


Figure 3. Humics Plus I_2 in NaOH - Methylene Iodide Plus Impurity

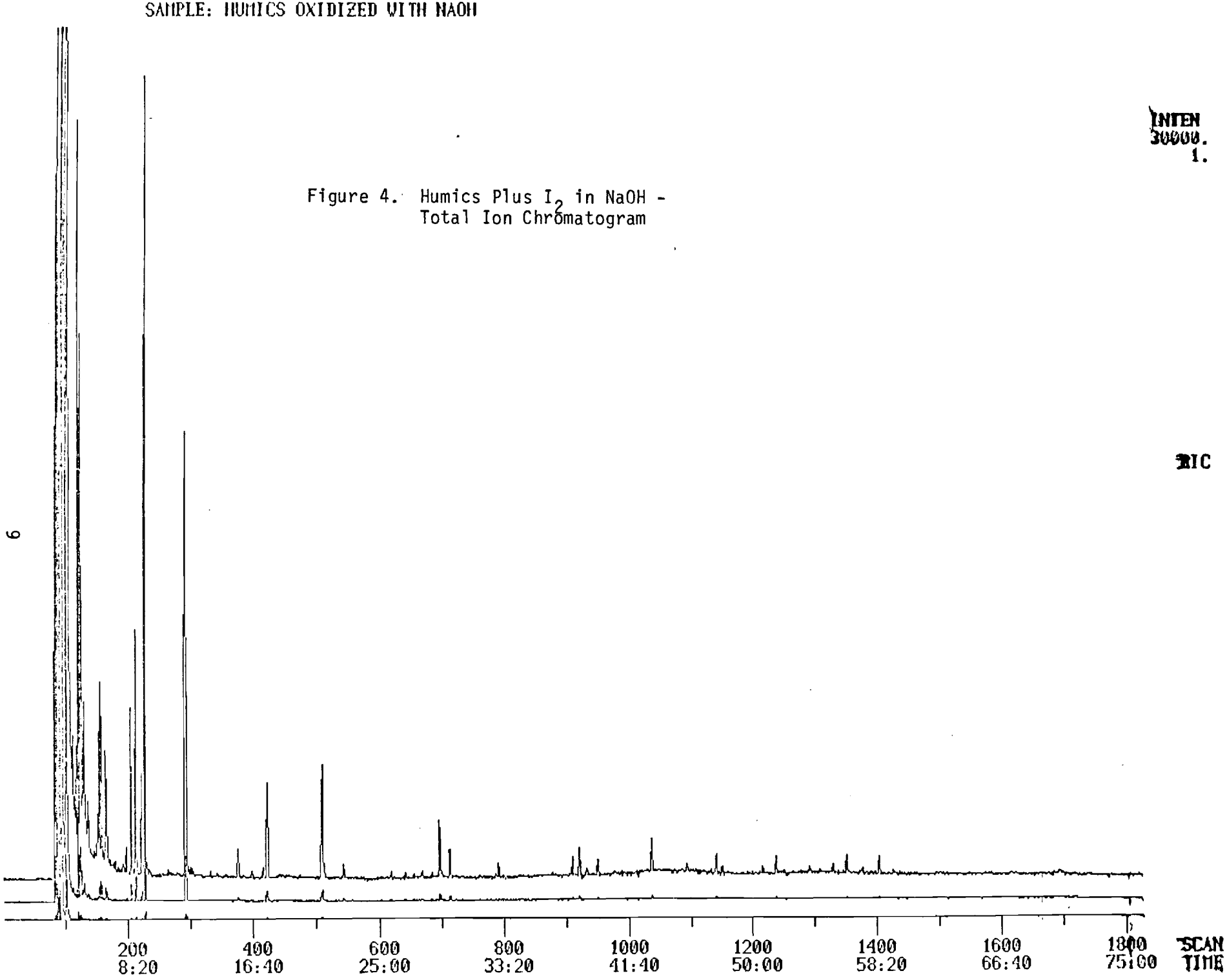


Figure 4. Humics Plus I_2 in NaOH - Total Ion Chromatogram

Table 1

Reaction Product Mixture -

Humics Oxidized with Iodine in Sodium Hydroxide

Scan No.	Probable Identity	Important Ions	Comments
102	Benzene	78, 63, 52, 41, 39	M^+, $C_5H_3^+$, $C_9H_4^+$, $C_3H_5^+$, $C_3H_3^+$ probably an artifact
104	Pyridine	79, 52	M^+, $C_4H_4^+$
	CH_3I ?	142, 127	M^+, I^+ needs to be resolved
	C_2H_5I	156, 127	M^+, I^+
112	3-methyl-2-pentanone possible mixed with 1,3-pentanediol	100, 71?, 57, 43	M^+, loss of C_2H_5, $C_4H_9^+$, $C_3H_7^+$
		118, 100, 87, 72, 43	M^+; loss of H_2O, CH_2OH, $CH_2OH + C_2H_4$; $C_3H_7^+$
118	Toluene plus impurities	92, 91	M^+, tropilium
121	ethyl isobutyrate?	116, 101, 87, 73, 71, 45, 43	M^+; loss of CH_3, C_2H_5, C_3H_7, OC_2H_5; $C_2H_5O^+$, $C_3H_7^+$
125	Butyl Acetate	101, 87, 73, 61, 56, 43, 41	loss of CH_3, C_2H_5, $C_3H_7^+$; $CH_3C(OH)_2^+$, $C_2H_5CH{=}CH_2$, $C_3H_5^+$
155	Ethyl Pentanoate	115, 101, 88, 85, 73, 70, 60, 57, 43, 41	loss of CH_3, C_2H_5, C_3H_6, OC_2H_5, C_4H_9, $C_3H_6 + H_2O$, $C_3H_6 + C_2H_4$, $C_4H_9^+$, $C_3H_7^+$, $C_3H_5^+$

Table 1 (Continued)

Scan No.	Probable Identity	Important Ions	Comments
165	Methylene Iodide (plus impurity)	268, 254, 141, 127	M^+, I_2^+, CH_2I^+, I^+ (see Figure 3)
198	2,2,4-trimethyl-3-hexanone	142, 100, 85, 57, 43, 41	M^+, loss of C_3H_6, C_4H_9; $C_4H_9^+$, $C_3H_5^+$ (see Figure 5)
212	Butyl Butyrate	116, 101, 89, 88, 73, 71, 56, 43, 41	loss of C_2H_4, C_3H_7; $C_3H_7\overset{+}{C}(OH)_2$; C_4H_8; $\overset{+}{O}C_4H_9$; $\overset{+}{O}C_7H_7$; $C_4H_8^+$; $C_3H_7^+$; $C_3H_5^+$

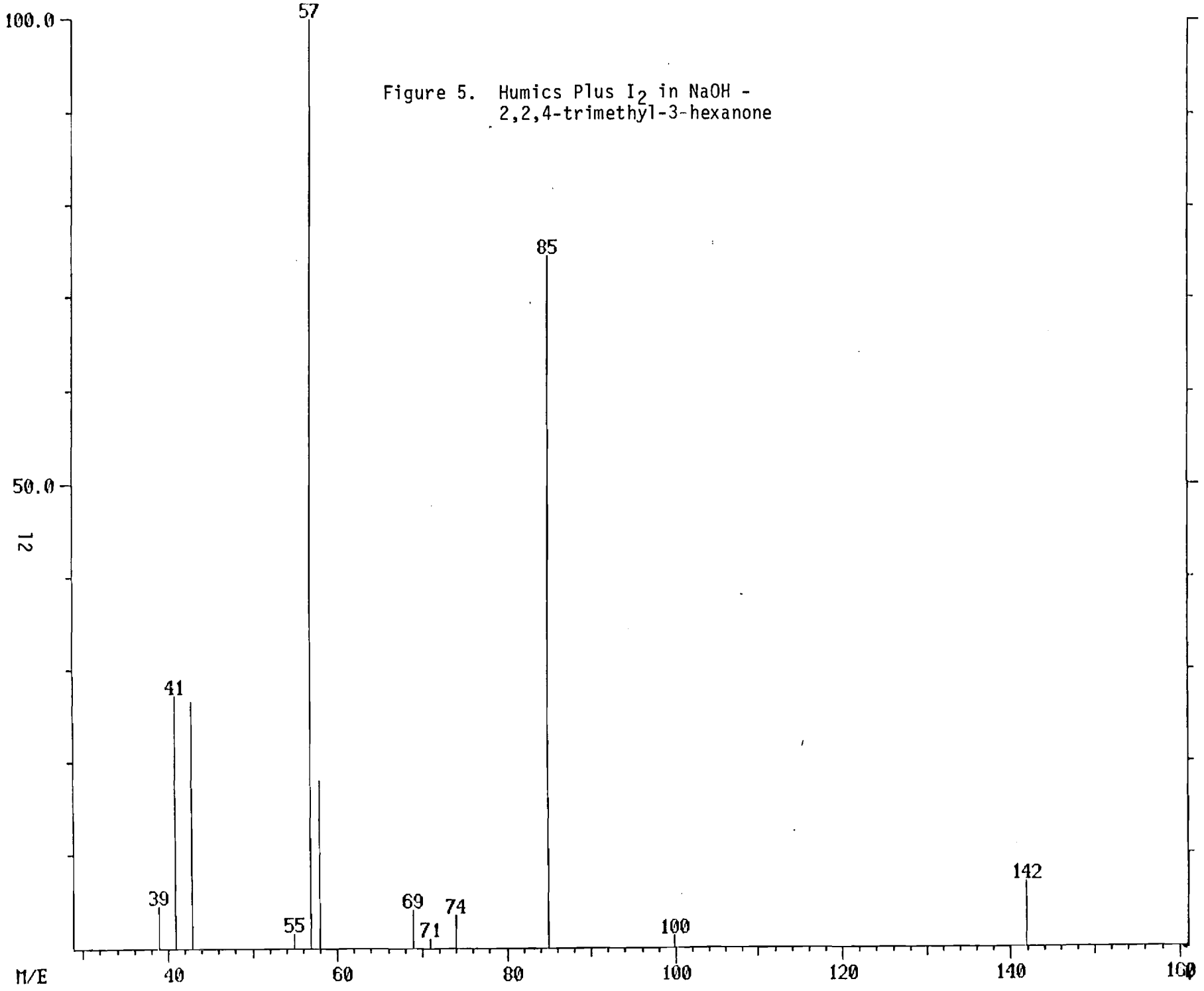


Figure 5. Humics Plus I_2 in NaOH - 2,2,4-trimethyl-3-hexanone

without addition of pH control reagents. The expectation was that flocculation would occur at some concentrations of inorganic flocculating agents as the pH was reduced below the levels used in the earlier experiments. Once conditions were determined for good flocculation, the pH at the point of optimum floc formation could then be controlled with the use of a buffer system. This is the way flocculation is normally optimized in a typical water treatment facility.

The results of jar test for flocculation of aquatic humic solutions using ferric chloride and aluminum sulfate, singly and in combination, are shown in Table 3.

After the observation of heavy flocculation in the mini-plant chlorination of M/30 aquatic humics using 100 mg of aluminum sulfate per liter of solution, a jar test using the same components and concentrations was conducted. A mixture of 1 L of purified water, 10 mg of M/30 aquatic humic material, 1.7 g potassium dihydrogen phosphate, 0.3 g of dipotassium hydrogen phosphate and 100 mg of aluminum sulfate was prepared and observed over a period of 18 hours. The mixture was slightly turbid; but no floc formation occurred. A solution of chlorine in 30 mL of purified water (prepared from 20 mL of commercial sodium hypochlorite solution and 10 mL of 1:3 sulfuric acid-water) was added to the jar and the contents mixed by swirling. A light floc formed one hour after addition of the chlorine-containing solution.

These results indicate that hypochlorous acid is required as a component of a mixture producing a floc with aluminum sulfate at a concentration of 100 mg/L. The aquatic humic material is probably partially oxidized by hypochlorous acid to provide ionic intermediates that combine with the aluminum hydroxide to produce particles of large enough size to settle out as a floc. The floc formed in the jar test was not as dense as that formed in the mini-plant.

It is apparent that the jar tests are not totally predictive of the degree of flocculation in mini-plant operation conditions. The difference between the jar test results and the mini-plant results is probably due to differences in the mixing conditions and the configuration of the glassware in which the floc formation occurs.

VII. REACTION OF AQUATIC HUMIC MATERIAL WITH CHLORINE-ANALYSIS OF PRODUCTS

It will be recalled that a sample of aquatic humic material was reacted with chlorine in the mini-pilot facility to provide a product

Table 2

Results of Jar Tests for Flocculation of Aquatic Humic Solutions

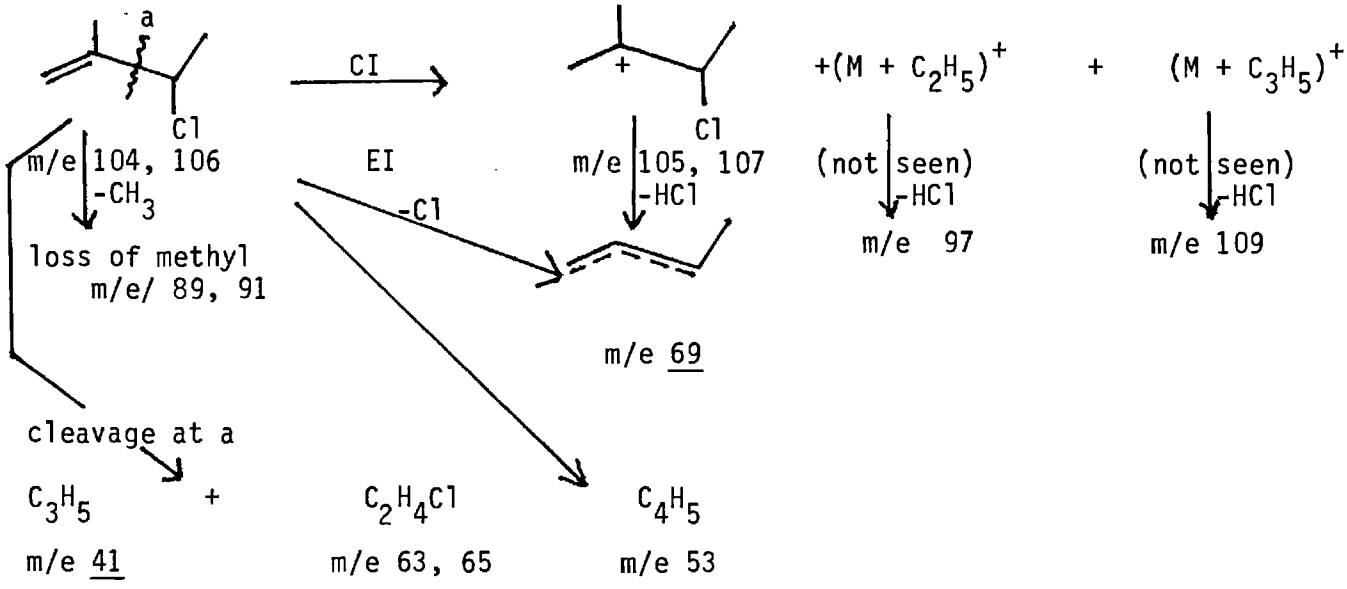
Run No.	Aquatic Humic Solid, mg	$Al_2(SO_4)_3$ Added, mg	$FeCl_3 \cdot 6H_2O$ Added, mg	pH	Appearance in Mixture
1	10	0	0	4.15	Clear
2	10	25	0	-	Clear
3	10	50	0	-	Clear
4	10	75	0	-	Clear
5	10	100	0	-	Clear
6	10	125	0	-	Clear
7	10	150	0	-	Clear
8	10	175	0	-	Clear
9	10	200	0	-	Clear
10	10	225	0	-	Clear
11	10	250	0	-	Clear
12	10	250	25	-	Clear
13	10	250	50	-	Clear
14	10	250	75	-	Clear
15	10	250	100	-	Turbid
16	10	250	125	-	Turbid
17	10	250	150	-	Turbid
18	10	250	175	-	Turbid
19	10	250	250	2.75	Turbid
20	10	0	0	4.05	Clear
21	10	0	25	-	Clear
22	10	0	50	-	Clear
23	10	0	75	-	Clear
24	10	0	100	-	Clear
25	10	0	125	-	Clear
26	10	0	150	-	Clear
27	10	0	175	-	Clear
28	10	0	200	-	Clear
29	10	0	225	-	Clear
30	10	0	250	-	Clear
31	10	25	250	-	Clear
32	10	50	250	-	Clear
33	10	75	250	-	Clear
34	10	100	250	-	Turbid
35	10	125	250	-	Turbid
36	10	175	250	2.8	Flocculation

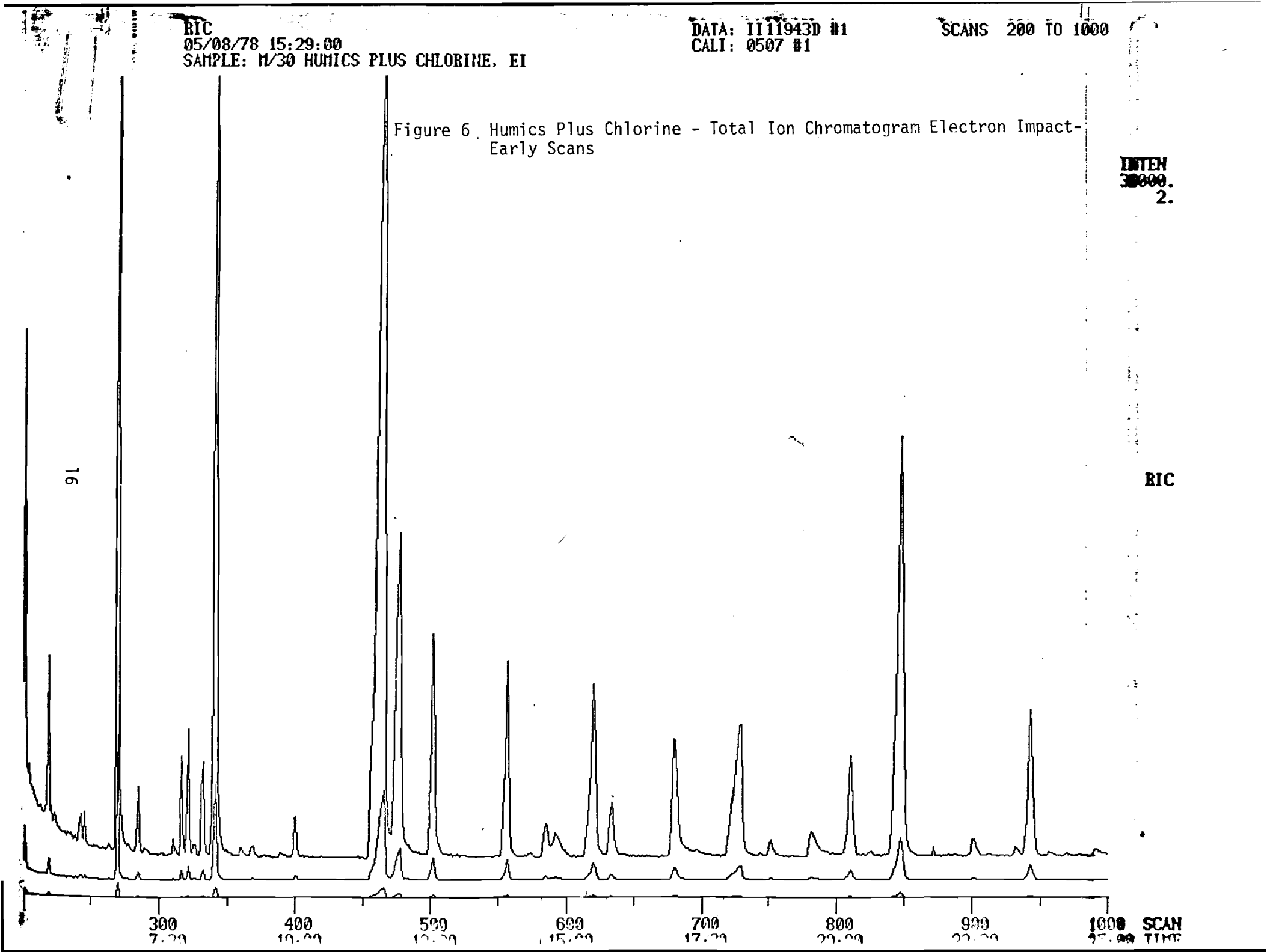
mixture which is rich in chlorinated organics (April report, Section IX, p. 25). While this reaction has now been repeated a number of times under slightly different conditions, the analysis has proceeded only on the original reaction mixture so that conditions might be optimized without risking the integrity of all of the samples in our possession.

Gas chromatographic separations have been improved by incorporating a ten-minute hold at the start of the temperature program. Achieving an exact match between the EI and CI chromatograms during the course of runs such as these which begin at low temperatures has proven to be more of a challenge than was originally anticipated. It has taken dozens of runs and a great deal of patience to produce the data which are described in the next few pages. The total ion chromatograms for the early scans are presented in Figures 6 and 7. The aforementioned difficulties with the mass assignments which now appears to have been resolved did not make this task easier. The products identified thus far will be systematically described below.

A. 3-Chloro-2-methyl-1-butene. The chlorinated isoprene structure shows a very good fit with library data with all fit indices over 900. The EI and CI spectra are consistent with one another as shown below. This peak was identified in the last report as the corresponding 2-ene.

The corresponding EI and CI spectra are presented in Figures 8 and 9 respectively.



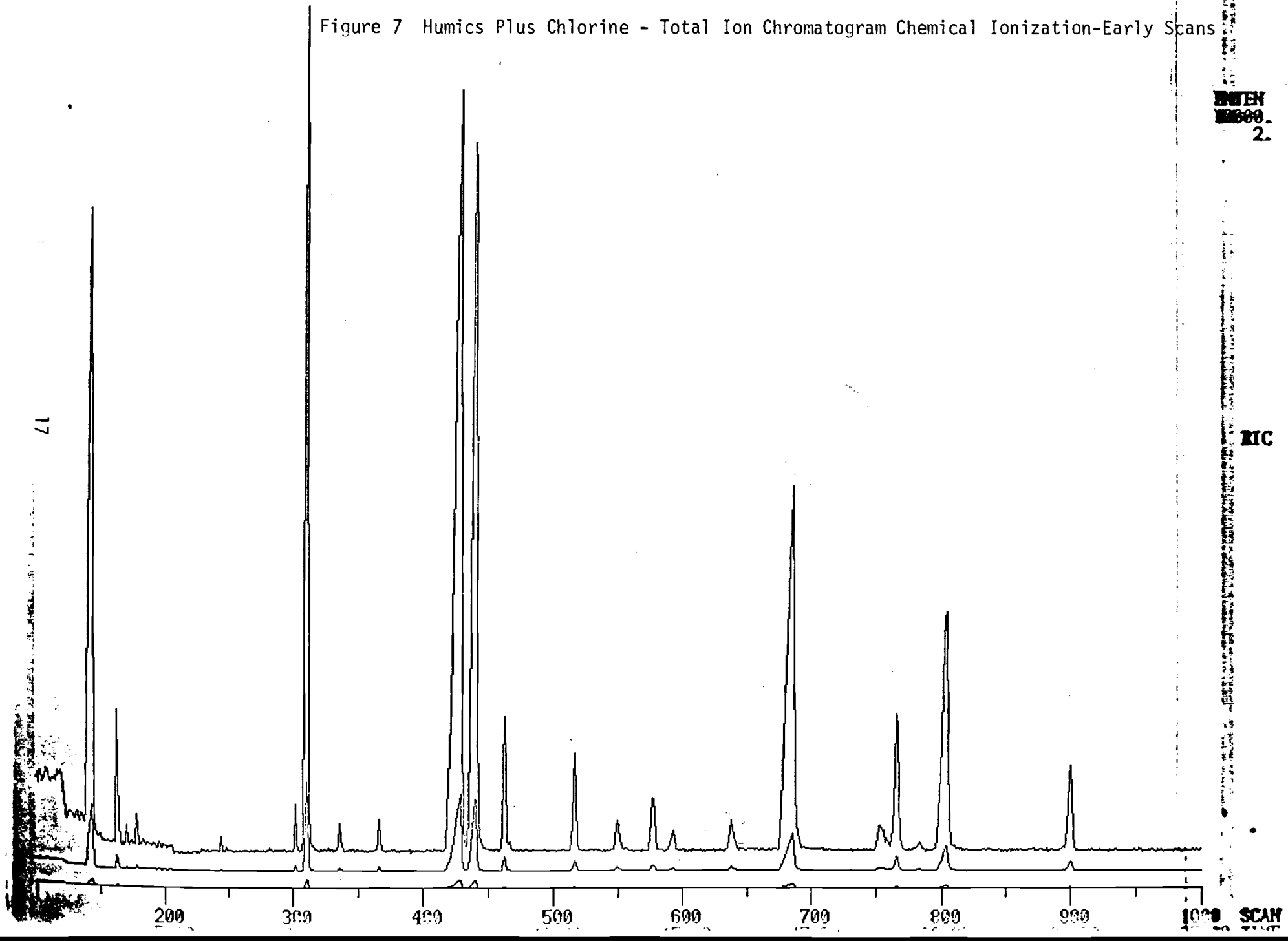

RIC
05/08/78 15:29:00
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI
DATA: II11943D #1
CALI: 0507 #1
SCANS 200 TO 1000
Figure 6. Humics Plus Chlorine - Total Ion Chromatogram Electron Impact-
Early Scans
INTEN
2.
RIC
91
300
400
500
600
700
800
900
1000
SCAN

RIC
05/09/78 9:56:00
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

DATA: I111943DCI #1
CALI: 0509 #3

SCANS 100 TO 1000

Figure 7 Humics Plus Chlorine - Total Ion Chromatogram Chemical Ionization-Early Scans



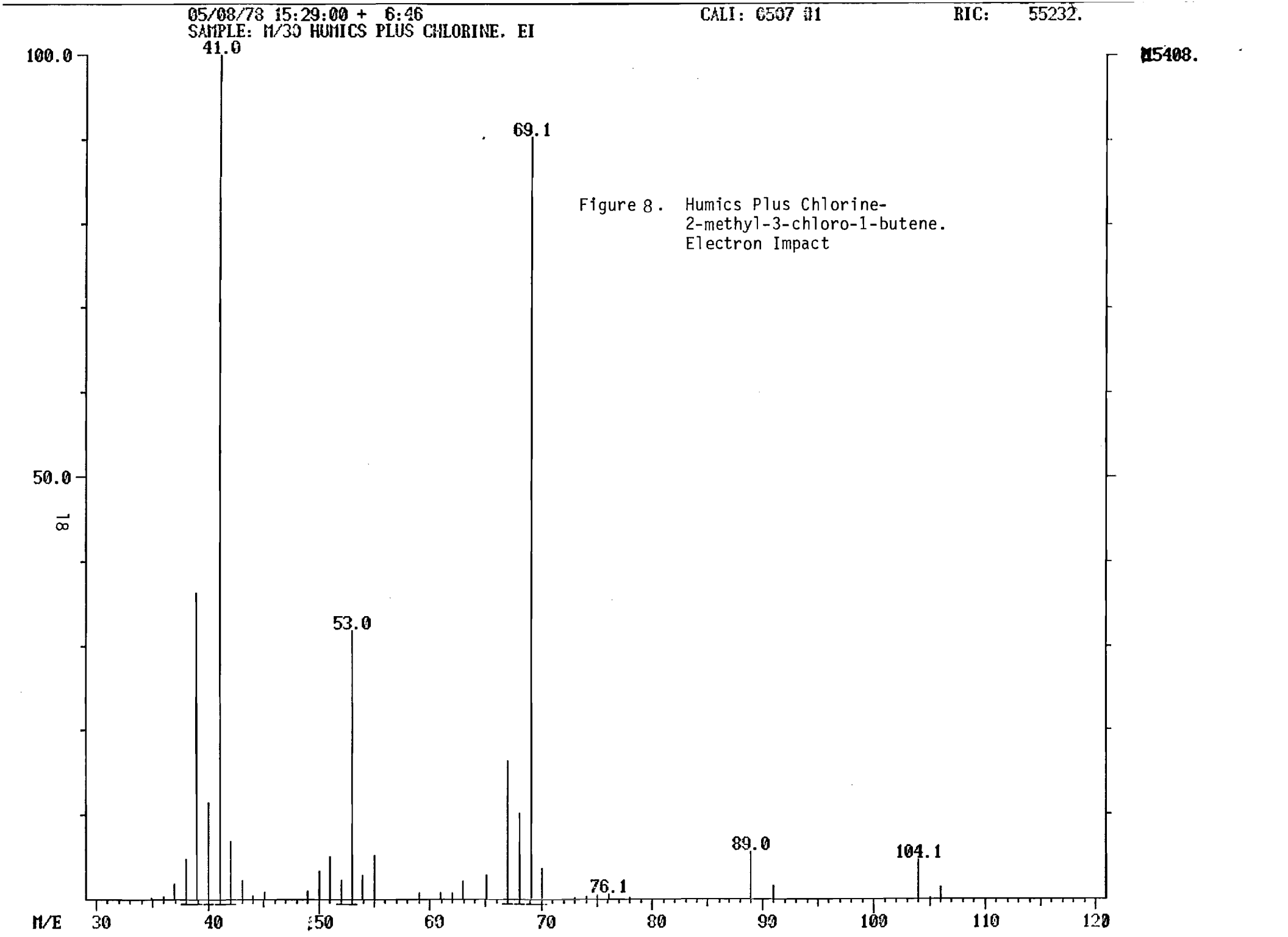


Figure 8. Humics Plus Chlorine-
2-methyl-3-chloro-1-butene.
Electron Impact

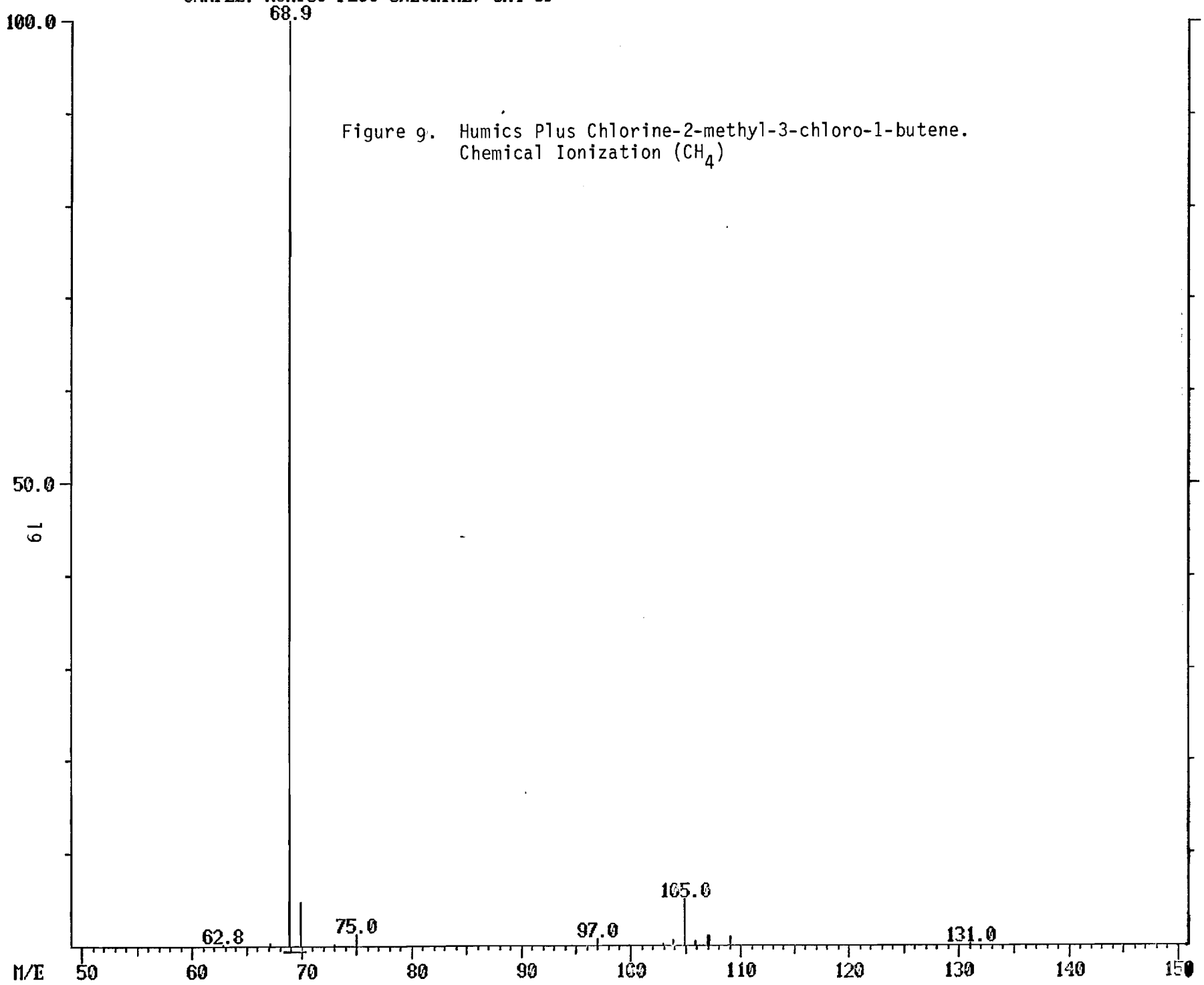
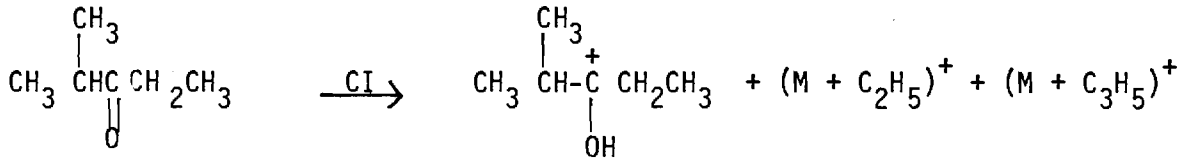


Figure 9. Humics Plus Chlorine-2-methyl-3-chloro-1-butene. Chemical Ionization (CH_4)

B. 2-methyl-3-pentanone. The second major non-hydrocarbon peak observed in the total ion chromatogram has been identified as 2-methyl-3-pentanone. The result of the library search showed another very good fit with all indicies being above 970. The fragmentation sequences are described below. The spectra are presented in Figures 10 and 11.



m/e 100 m/e 101 m/e/ 129 m/e 141

EI

loss of methyl + loss of ethyl + loss of isopropyl

m/e 85 m/e 71 m/e 57

$C_3H_7^+$ + $C_3H_5^+$ + $C_3H_3^+$

m/e 43 m/e 41 m/e 39

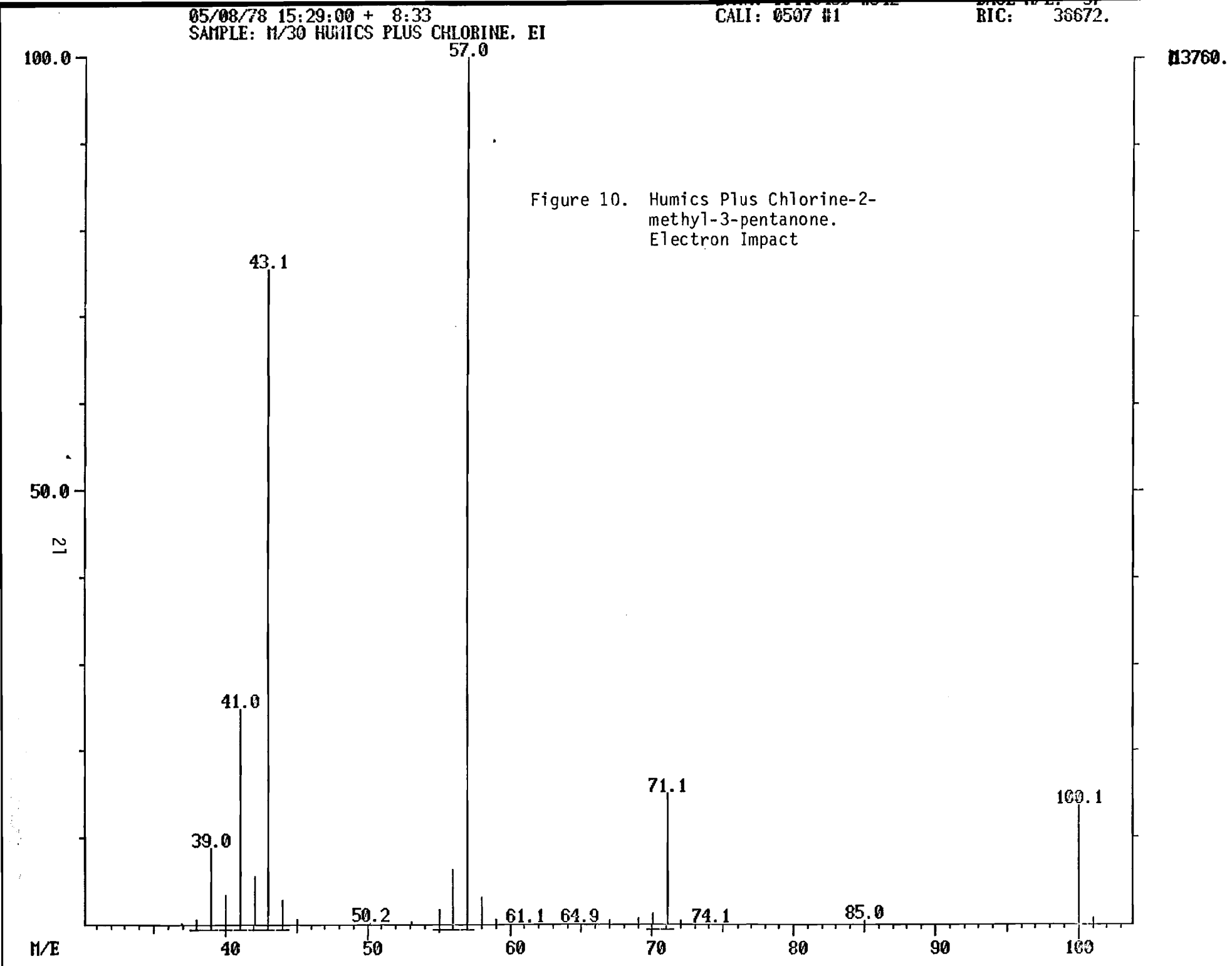


Figure 10. Humics Plus Chlorine-2-methyl-3-pentanone. Electron Impact

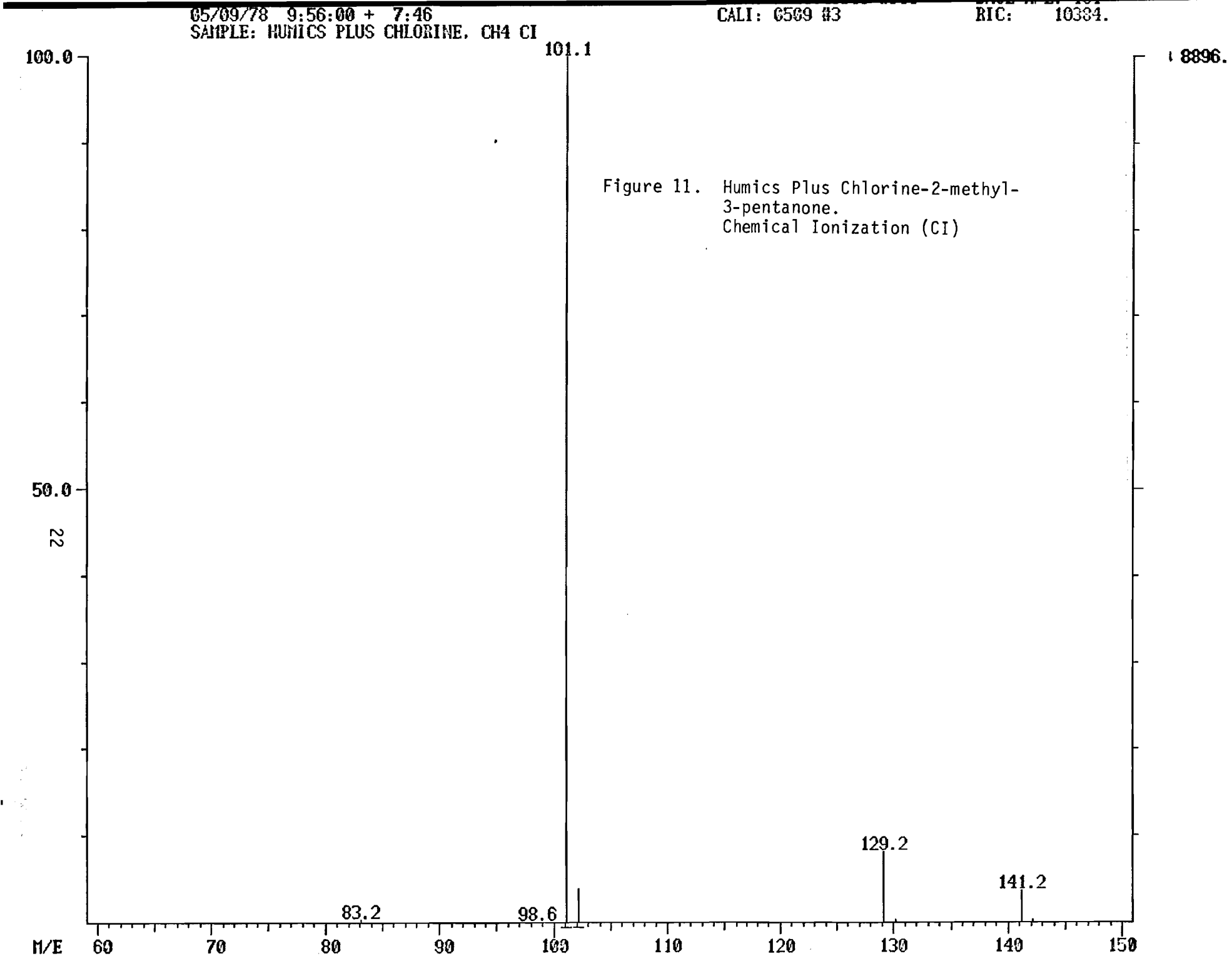
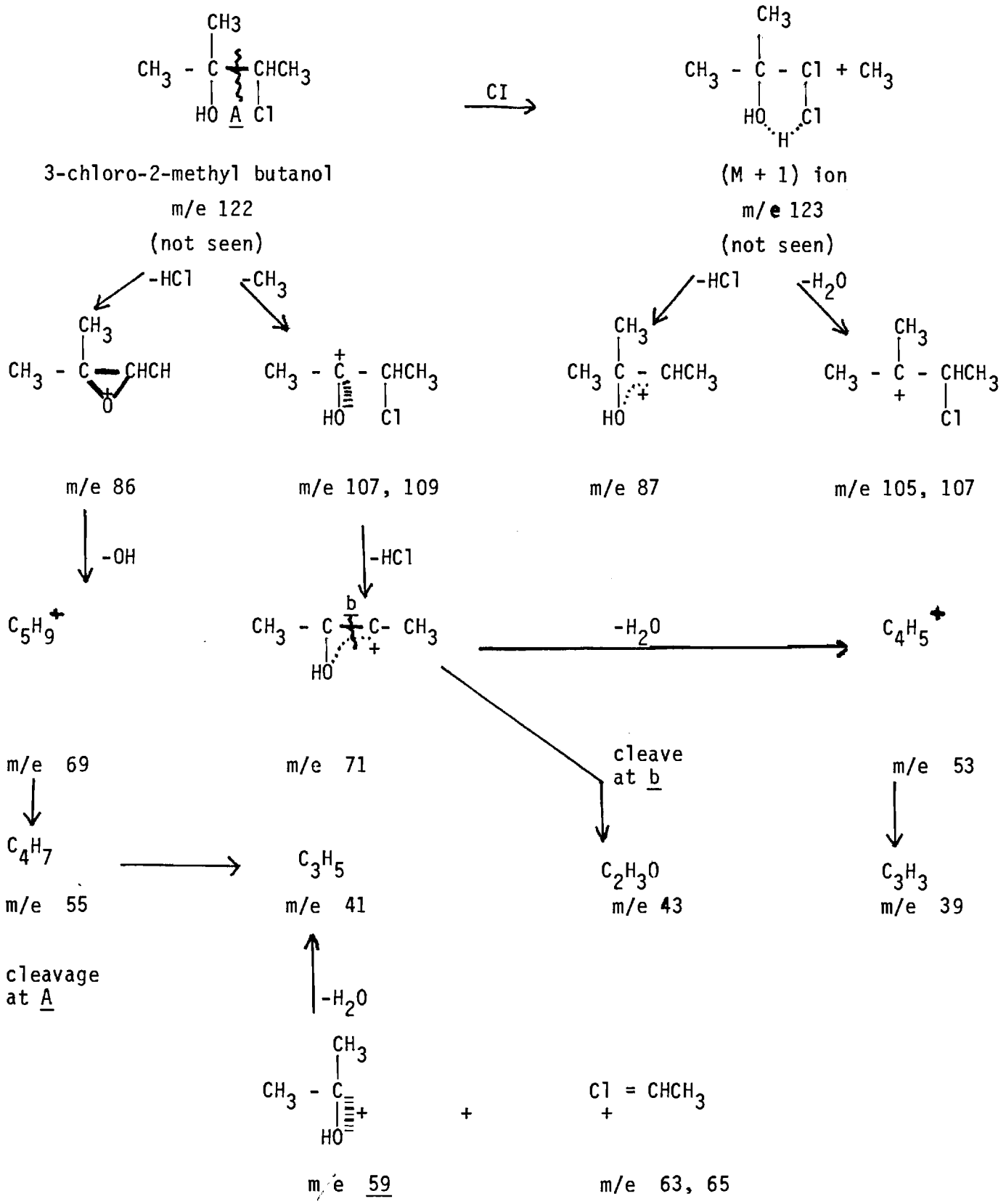


Figure 11. Humics Plus Chlorine-2-methyl-3-pentanone.
Chemical Ionization (CI)

C. Unknown "nitrogen" compound. The CI data have persuaded the writers to change their minds regarding the presence of nitrogen in this compound. After considerable effort, the staff has been able to postulate a structure which does not contain nitrogen but which is consistent with both the EI and CI fragmentation patterns which are presented in Figures 12 and 13. The fact that the postulated structure has the same arrangement of carbon and chlorine atoms as does the previously identified 3-chloro-2-methyl butene adds further strength to the assignment. Since a library "hit" was not achieved, absolute confirmation of the structure would depend on obtaining or synthesizing an authentic sample. A proposed mechanism explaining the observed fragmentation is outlined on the next page. The structural assignment made is 3-chloro-2-methyl-2-butanol.


3-chloro-2-methyl butanol
m/e 122
(not seen)
CI
(M + 1) ion
m/e 123
(not seen)
-HCl
-CH3
-HCl
-H2O
m/e 86
m/e 107, 109
m/e 87
m/e 105, 107
-OH
-HCl
-H2O
C5H9+
C4H5+
m/e 69
m/e 71
cleave at b
m/e 53
C4H7
C3H5
C2H3O
C3H3
m/e 55
m/e 41
m/e 43
m/e 39
cleavage at A
-H2O
m/e 59
m/e 63, 65

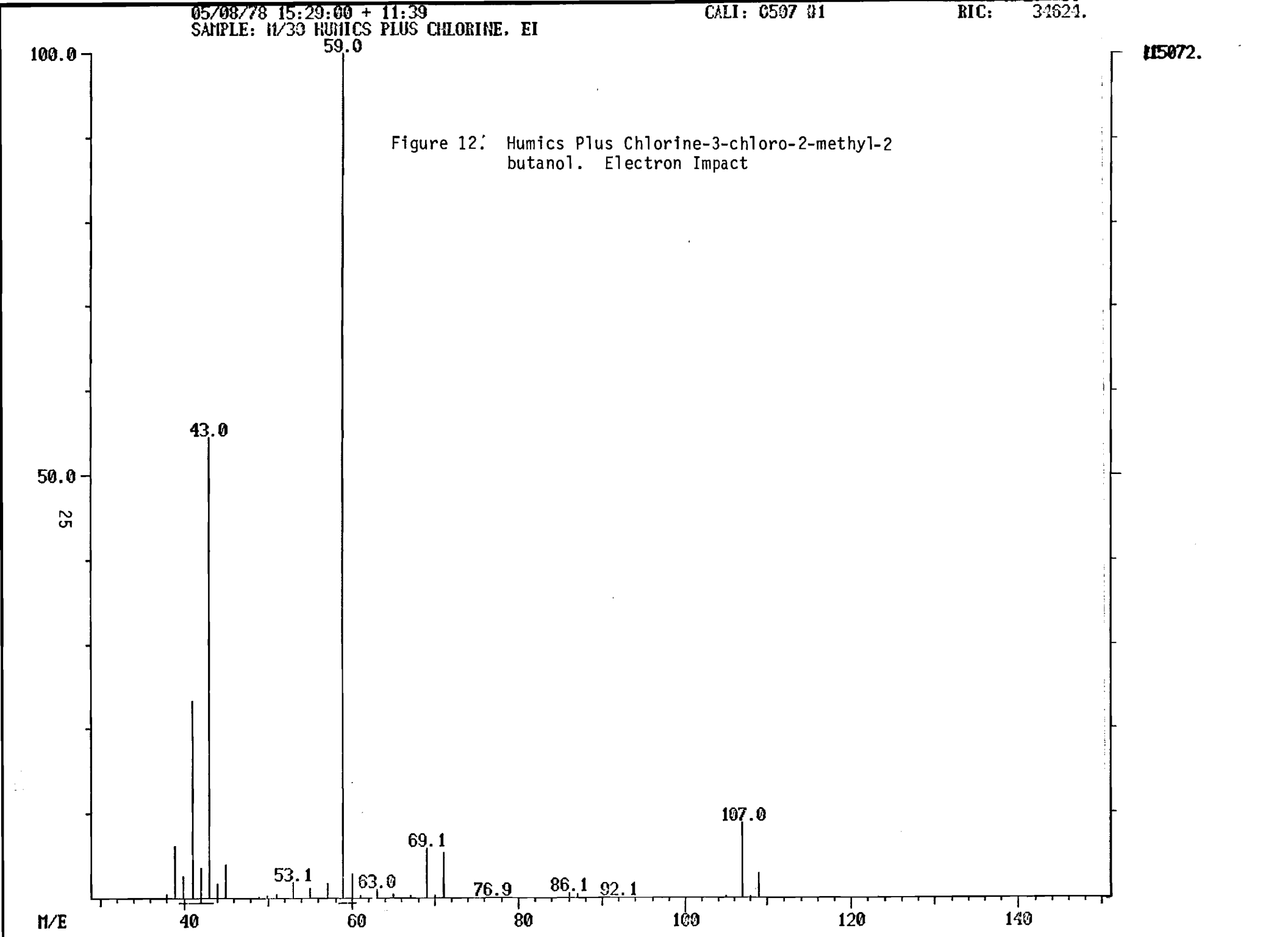


Figure 12. Humics Plus Chlorine-3-chloro-2-methyl-2 butanol. Electron Impact

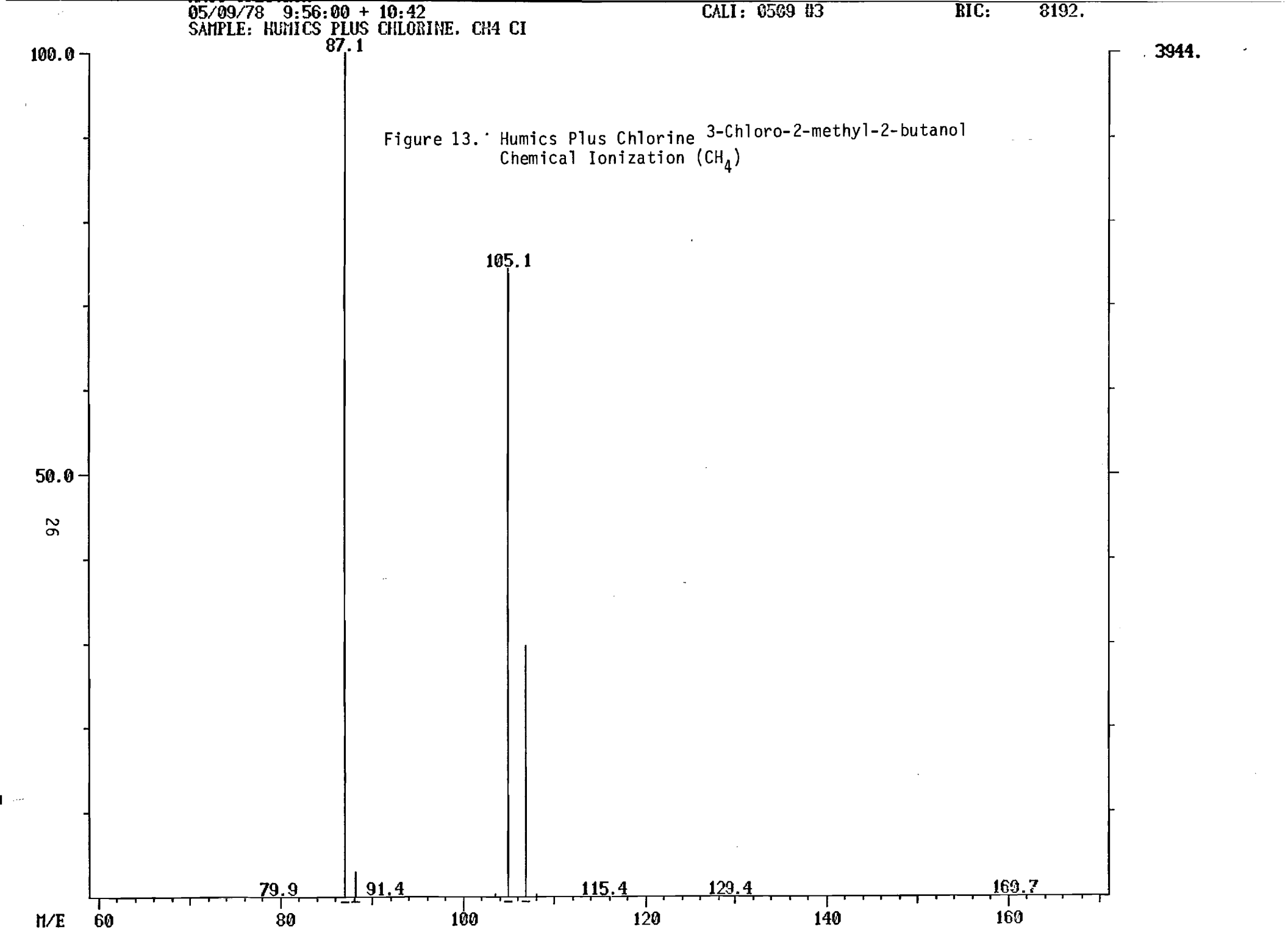
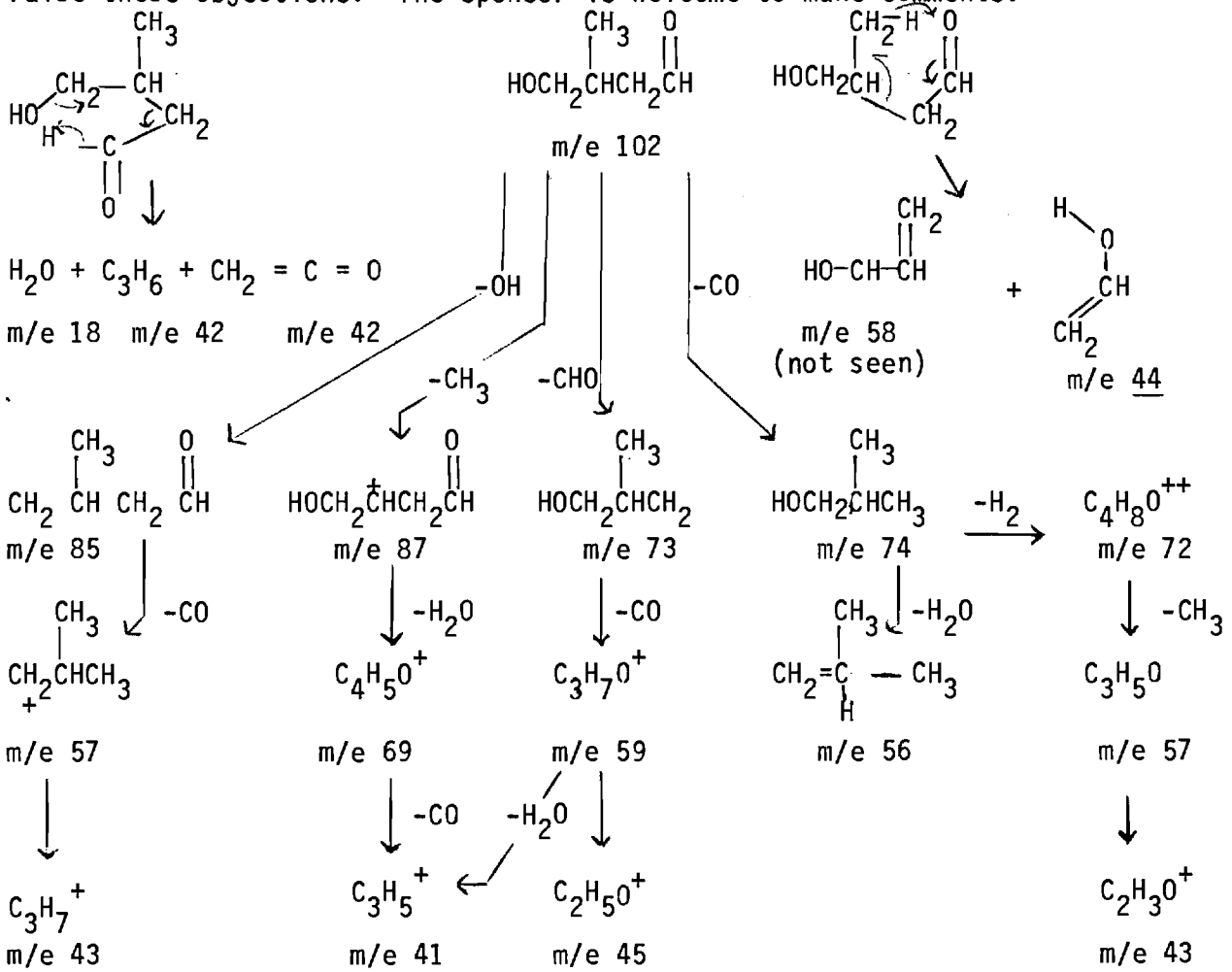


Figure 13. Humics Plus Chlorine 3-Chloro-2-methyl-2-butanol
Chemical Ionization (CH_4)

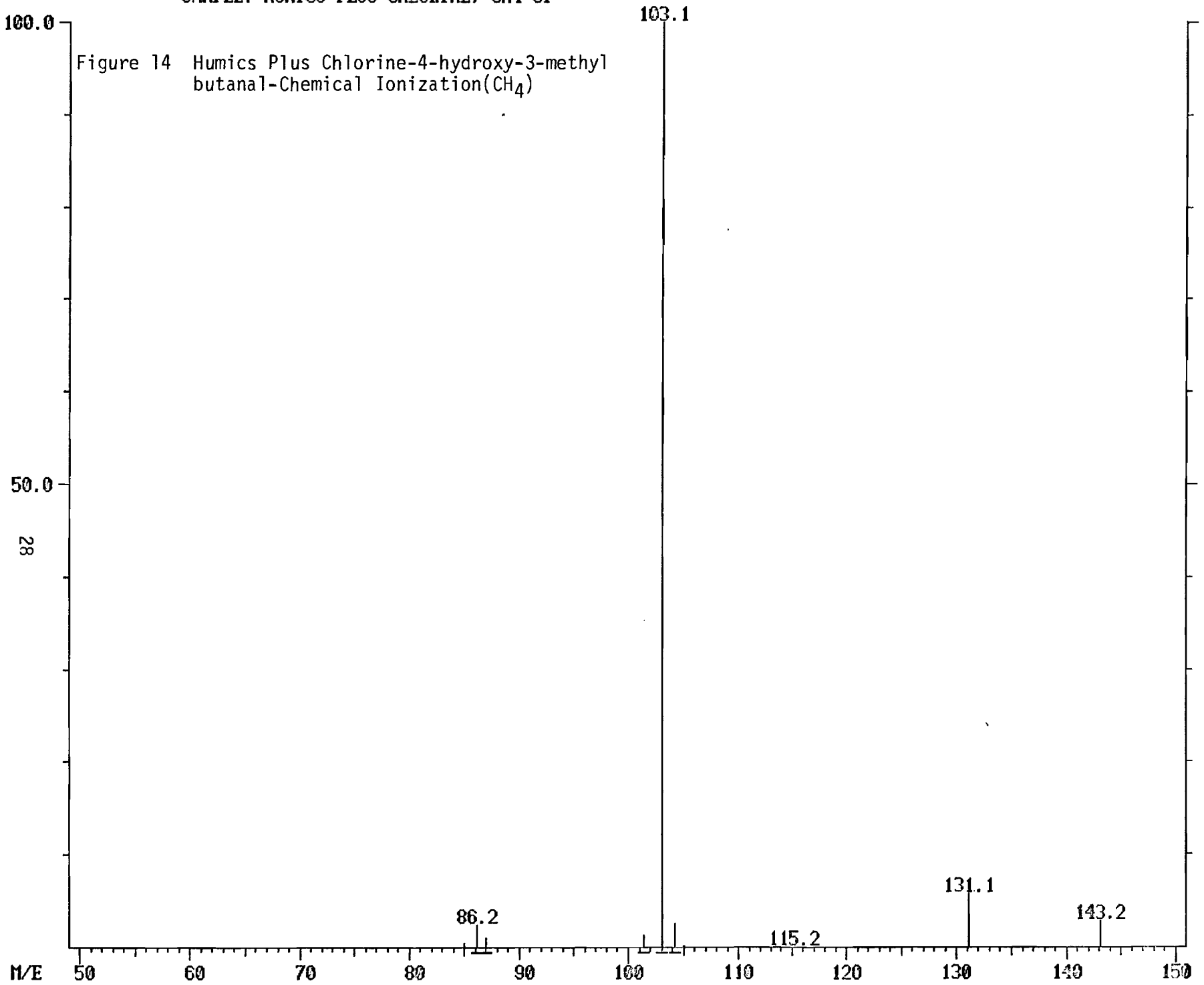
D. 4-hydroxy-3-methyl butanal. The chemical ionization mass spectrum shown in Figure 14 with ions at m/e 103 (M + 1), 131 (M + 29) and 143 (M + 41) fixes the molecular weight at 102. In addition, a weak M-1 ion and loss of OH to provide a fragment ion at 86 suggested that an alcoholic function was present.

The electron impact spectrum supported this evidence (see Figure 15). The key ion in the fragmentation pattern is seen at m/e 44 (base peak) and can be accounted for by invoking the McLafferty rearrangement as shown in the upper right hand corner of the mechanistic scheme outlined below. Although other isomers may also explain the observed fragmentation pattern, the writers have elected to favor the isoprenoid structures out of biogenetic considerations. We are not entirely happy with the structure since it is not obvious why the oxidation reaction should have stopped at the aldehyde stage (hemiacetal formation?), or why dehydration does not occur; it may be that some other structure will be found which does not raise these objections. The sponsor is welcome to make comments.



05/09/78 9:56:00 + 11:00 CALI: 0509 #3 RIC: 8352.
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

Figure 14 Humics Plus Chlorine-4-hydroxy-3-methyl butanal-Chemical Ionization(CH_4)



05/08/78 15:29:00 + 11:57 CALI: 0507 01 RIC: 12576.
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI

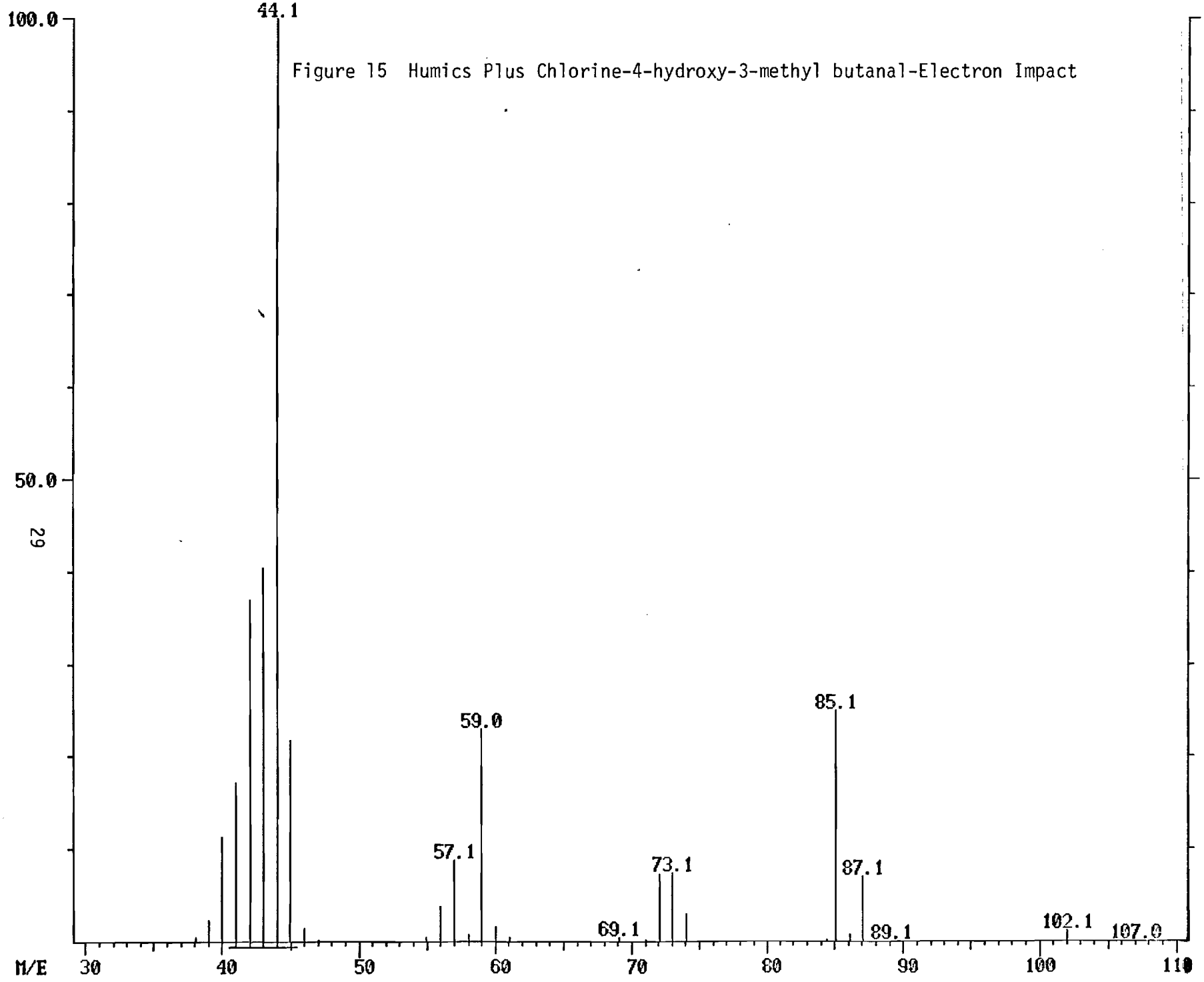
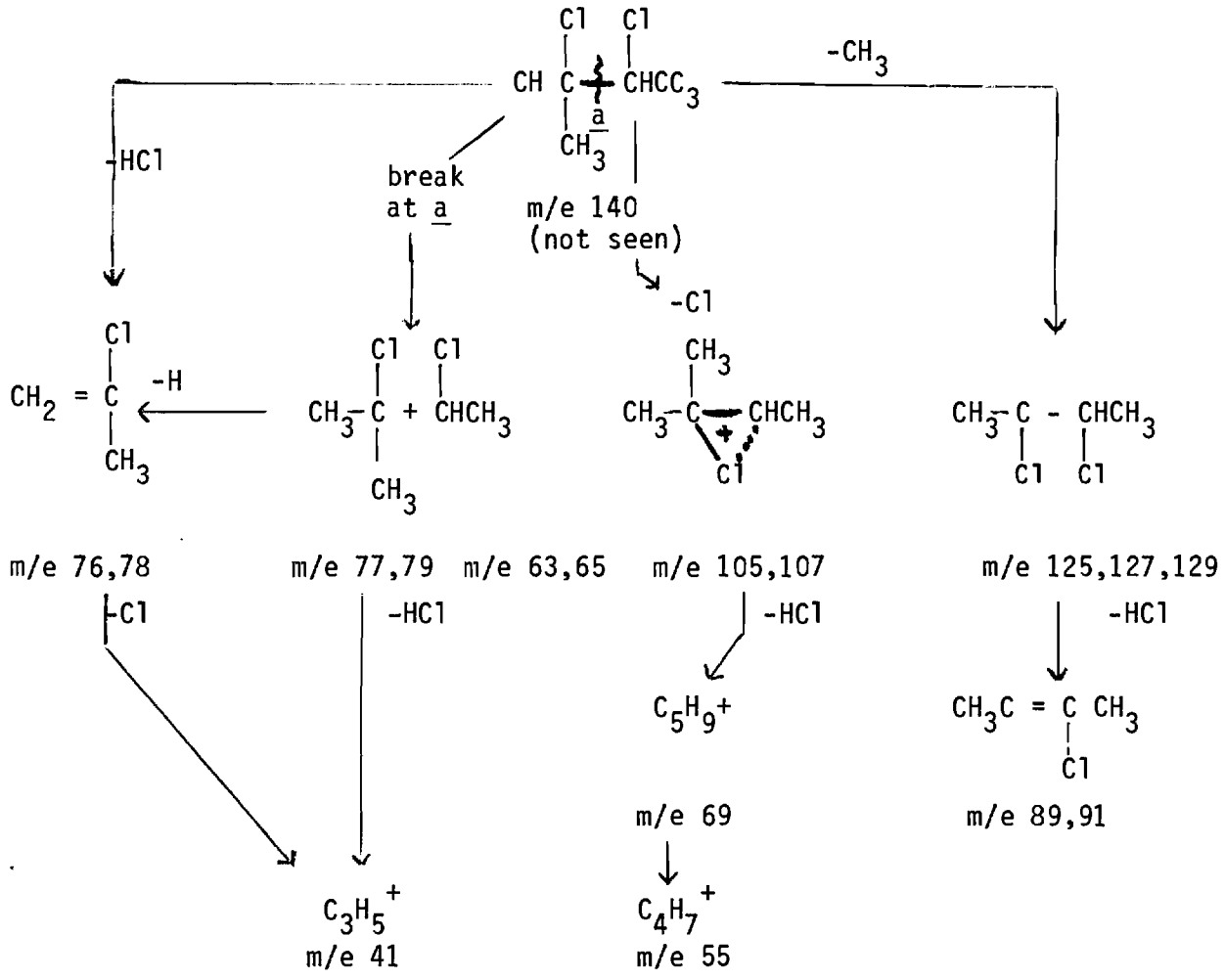
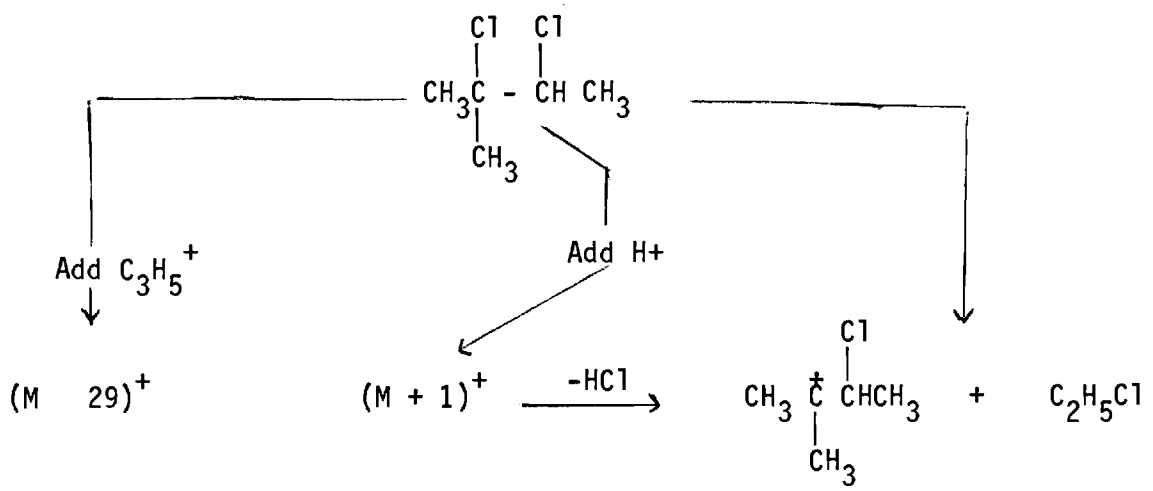


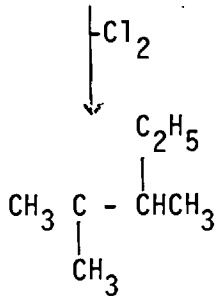
Figure 15 Humics Plus Chlorine-4-hydroxy-3-methyl butanal-Electron Impact

E. 2,3-dichloro-2-methylbutane. The identity of this compound was fairly well established in the last monthly report. A more complete interpretation of the electron impact fragmentation pattern is presented below. The spectrum itself is presented in Figure 16.



The methane chemical ionization spectrum like that already observed in the case of the postulated 3-chloro-2-methyl-2-butanol does not show the expected M+1, $M+C_2H_5$, $M+C_3H_5$ pattern. Instead, a loss of HCl from M+1 or a displacement of chlorine as ethyl chloride by $C_2H_5^+$ produces the base peak at m/e 105. The fragmentation pattern is outlined on the next page. The common substitution pattern observed in the products thus far lends further strength to each assignment and suggests a common origin. The bar graph is presented in Figure 17.


Cl Cl
CH3C - CH CH3
CH3
Add C3H5+
Add H+
(M 29)+
(M + 1)+
-HCl
Cl
CH3 C CHCH3
CH3
+
C2H5Cl
m/e 169
(not seen)
m/e 141
(not seen)
m/e 105,107


Cl2
C2H5
CH3 C - CHCH3
CH3

m/e 99

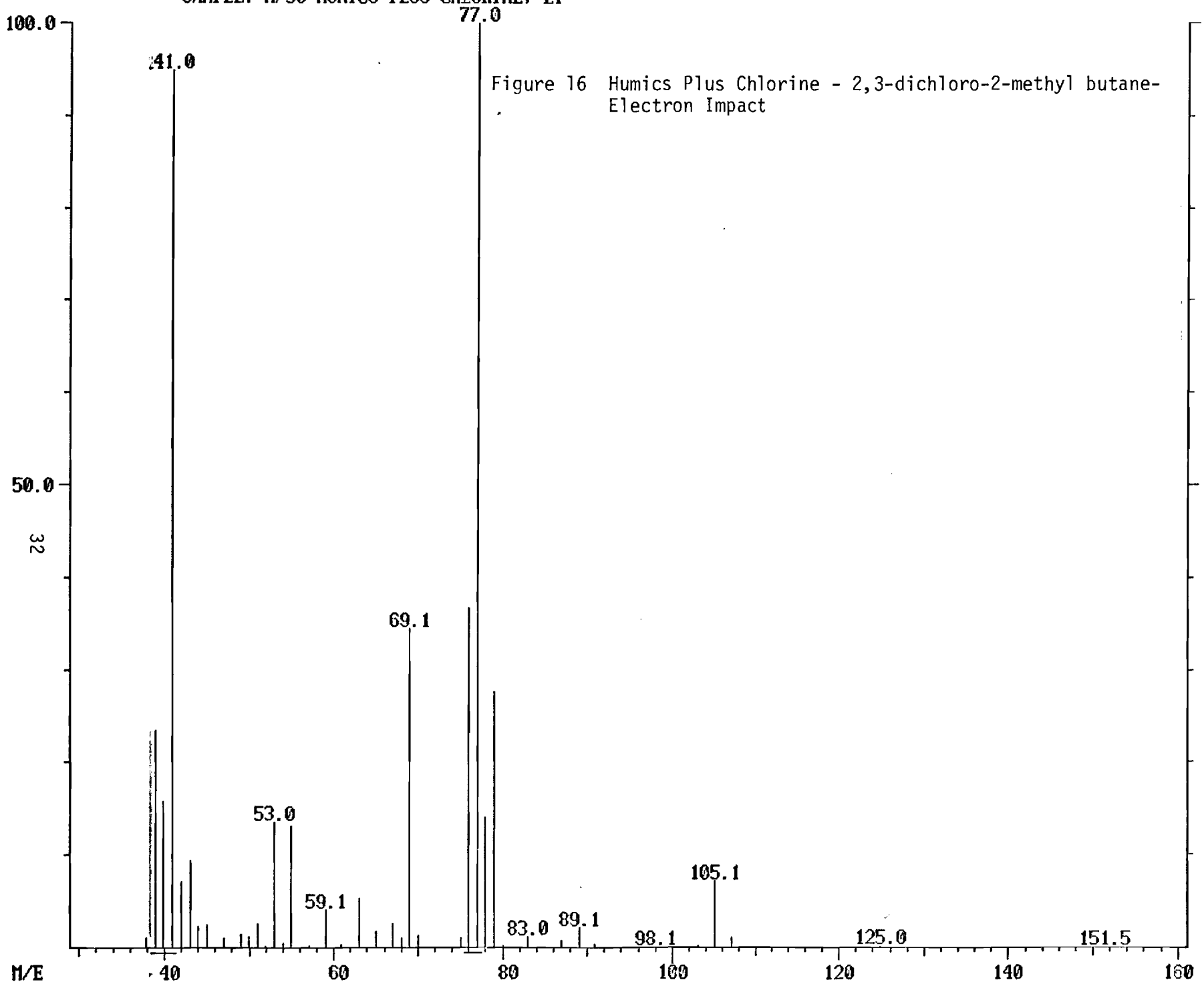


Figure 16 Humics Plus Chlorine - 2,3-dichloro-2-methyl butane-
Electron Impact

05/09/78 9:56:00 + 11:34 CALI: 0509 #3 RIC: 1626.
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

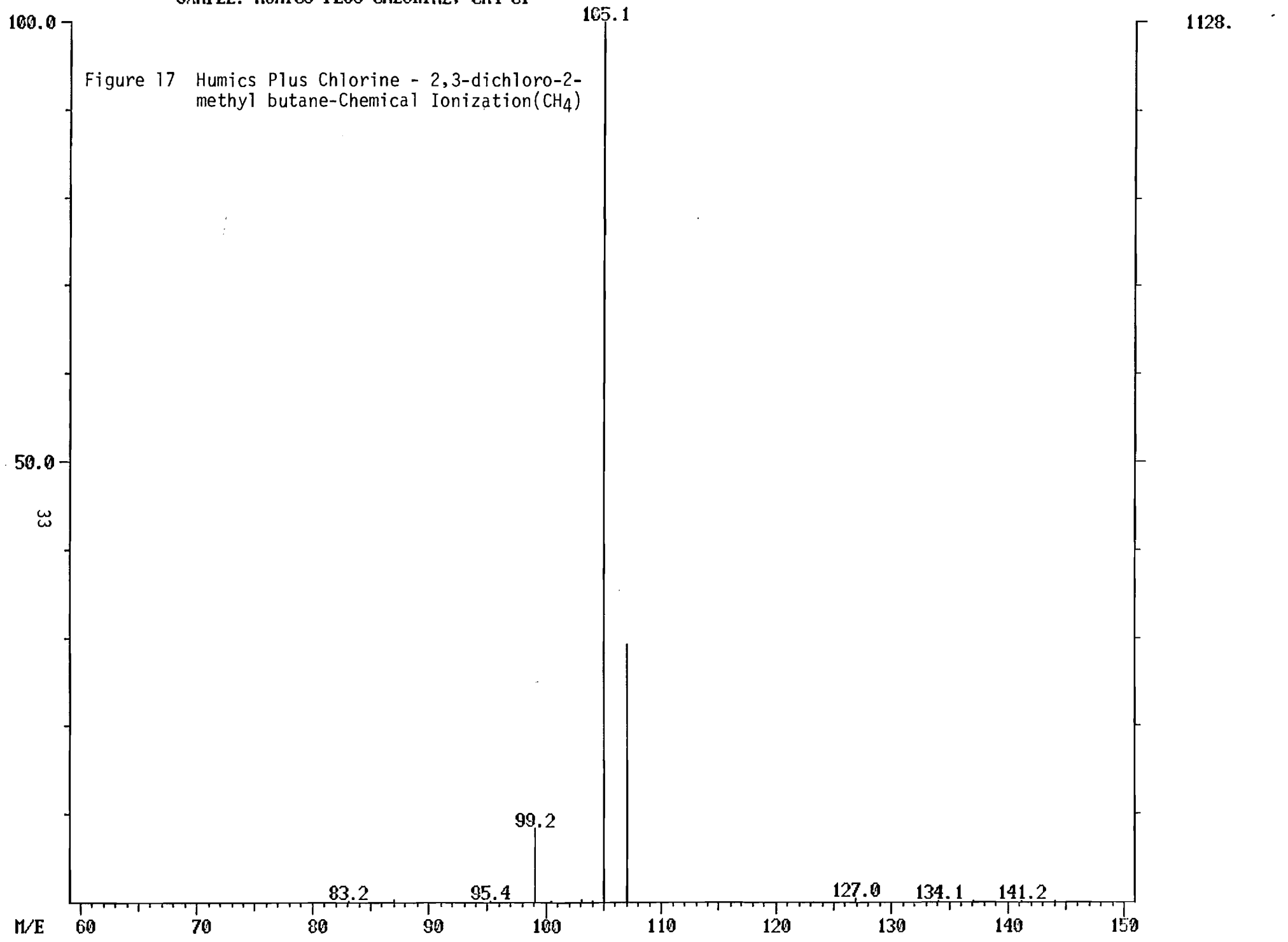
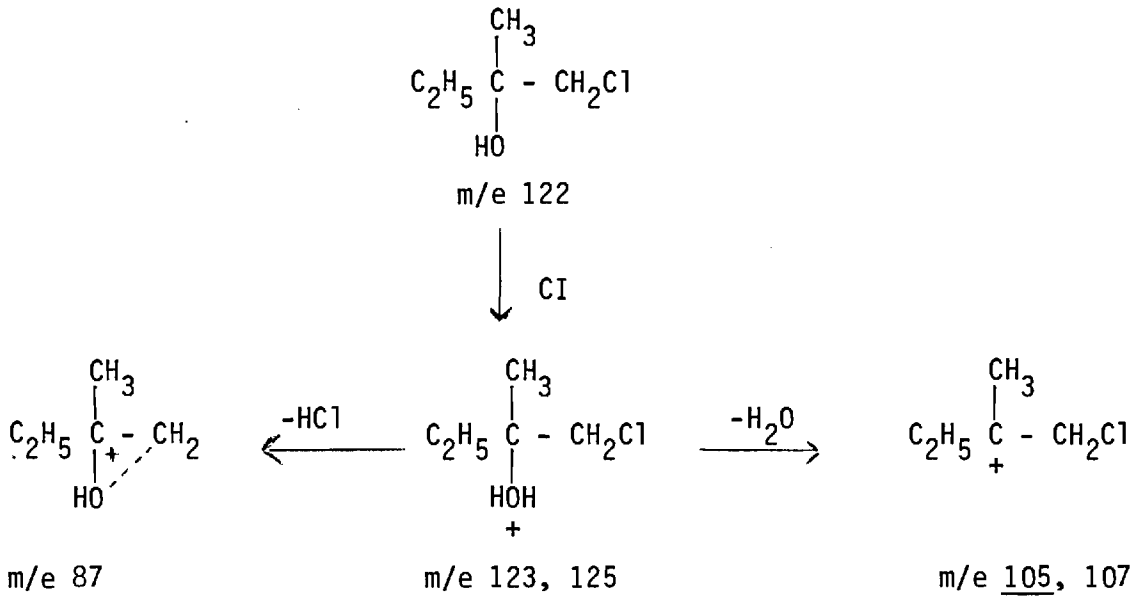
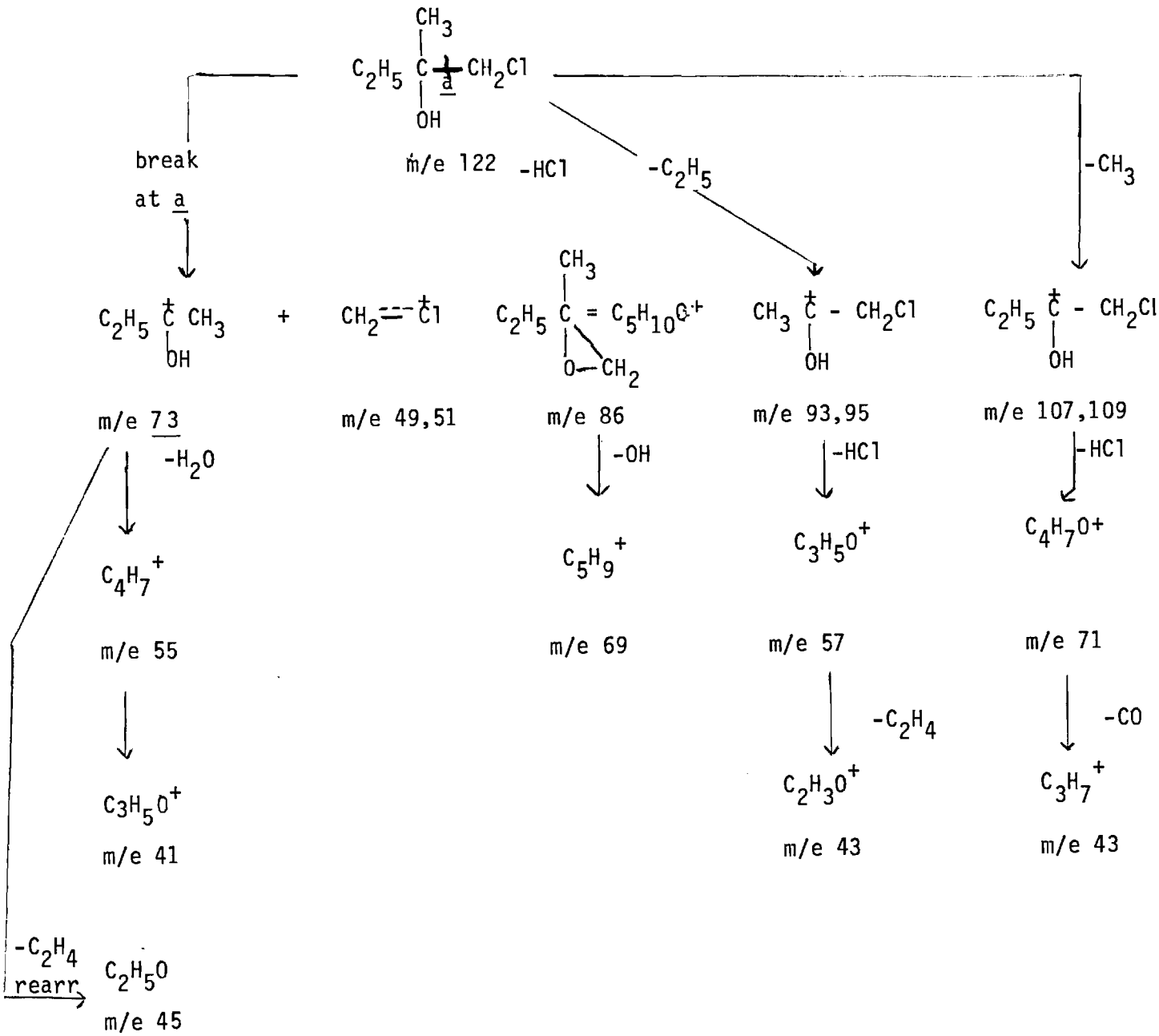


Figure 17 Humics Plus Chlorine - 2,3-dichloro-2-methyl butane-Chemical Ionization(CH_4)

F. 1-chloro-2-methyl-2-butanol. It will be noted that this compound is another example of an "unknown nitrogen-chlorine compound" turning out to be a non-nitrogenous substance upon examination of the CI data. The peaks at 107 and 109 in the EI fragmentation patterns and the entire CI pattern strongly suggests that this material is an isomer of compound C. The CI results which are outlined below and presented in Figure 18 suggest that loss of water should be more favorable and loss of hydrochloric acid less favorable than before. On this basis, the indicated structural assignment was made. In this case, a weak quasi-molecular ion was observed at m/e 123.



The electron impact data which are presented in Figure 19 are supportive of this assignment. A fragmentation mechanism which accounts for all major ions is outlined on the next page.


CH3
C2H5 C a CH2Cl
OH
m/e 122
-HCl
-C2H5
-CH3
break at a
C2H5 C CH3
OH
+
CH2=Cl
C2H5 C = C5H10O+
O—CH2
CH3 C - CH2Cl
OH
C2H5 C - CH2Cl
OH
m/e 73
-H2O
m/e 49,51
m/e 86
-OH
m/e 93,95
-HCl
m/e 107,109
-HCl
C4H7+
m/e 55
C5H9+
m/e 69
C3H5O+
m/e 57
C4H7O+
m/e 71
-C2H4
-CO
C3H5O+
m/e 41
C2H2O+
m/e 43
C3H7+
m/e 43
-C2H4
rearr
C2H5O
m/e 45

05/09/78 9:56:00 + 12:55 CALI: 0569 #3 RIC: 1198.
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

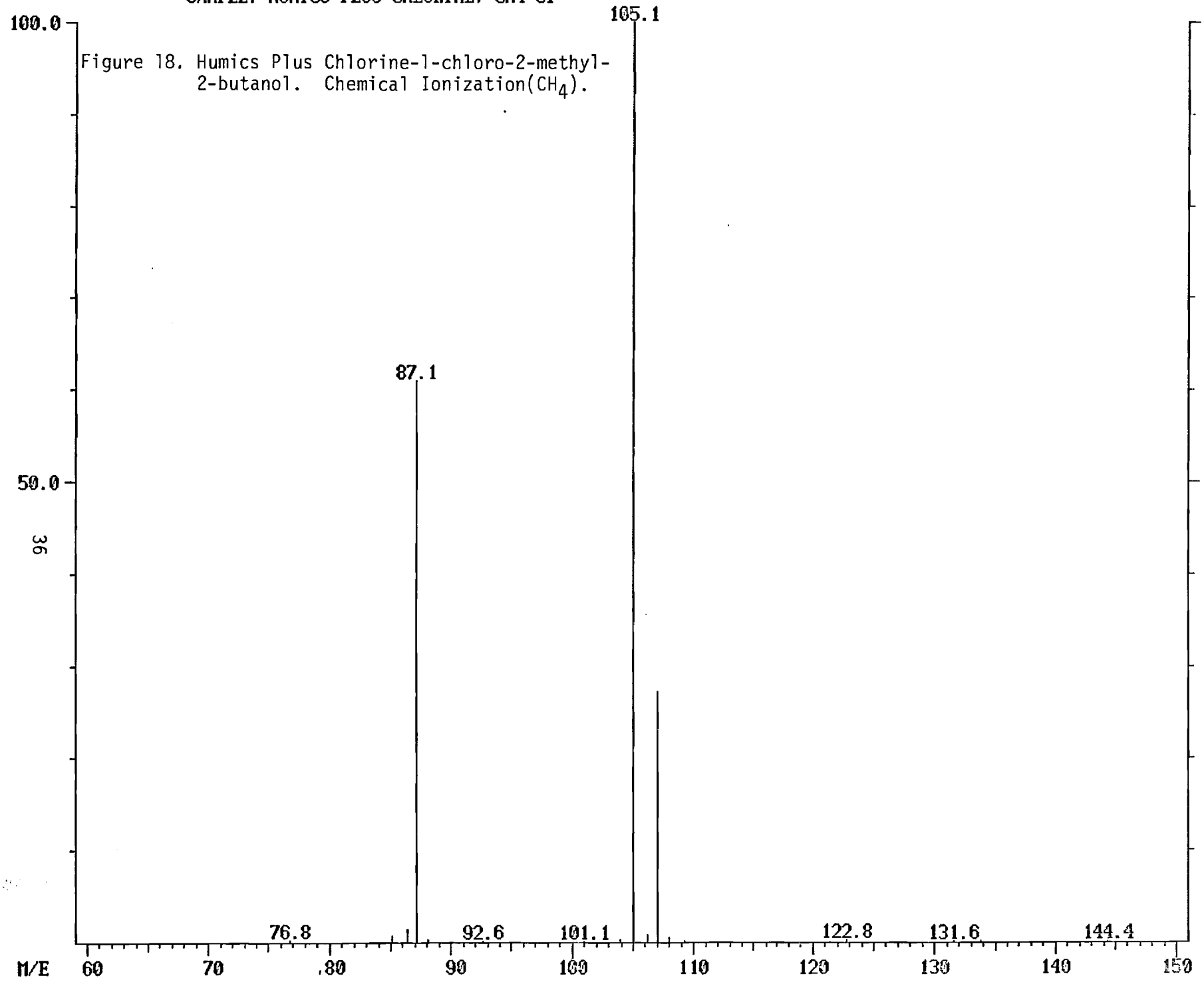


Figure 18. Humics Plus Chlorine-1-chloro-2-methyl-2-butanol. Chemical Ionization(CH_4).

05/08/78 15:29:00 + 13:55 CALI: 0507 01 RIC: 7768.
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI

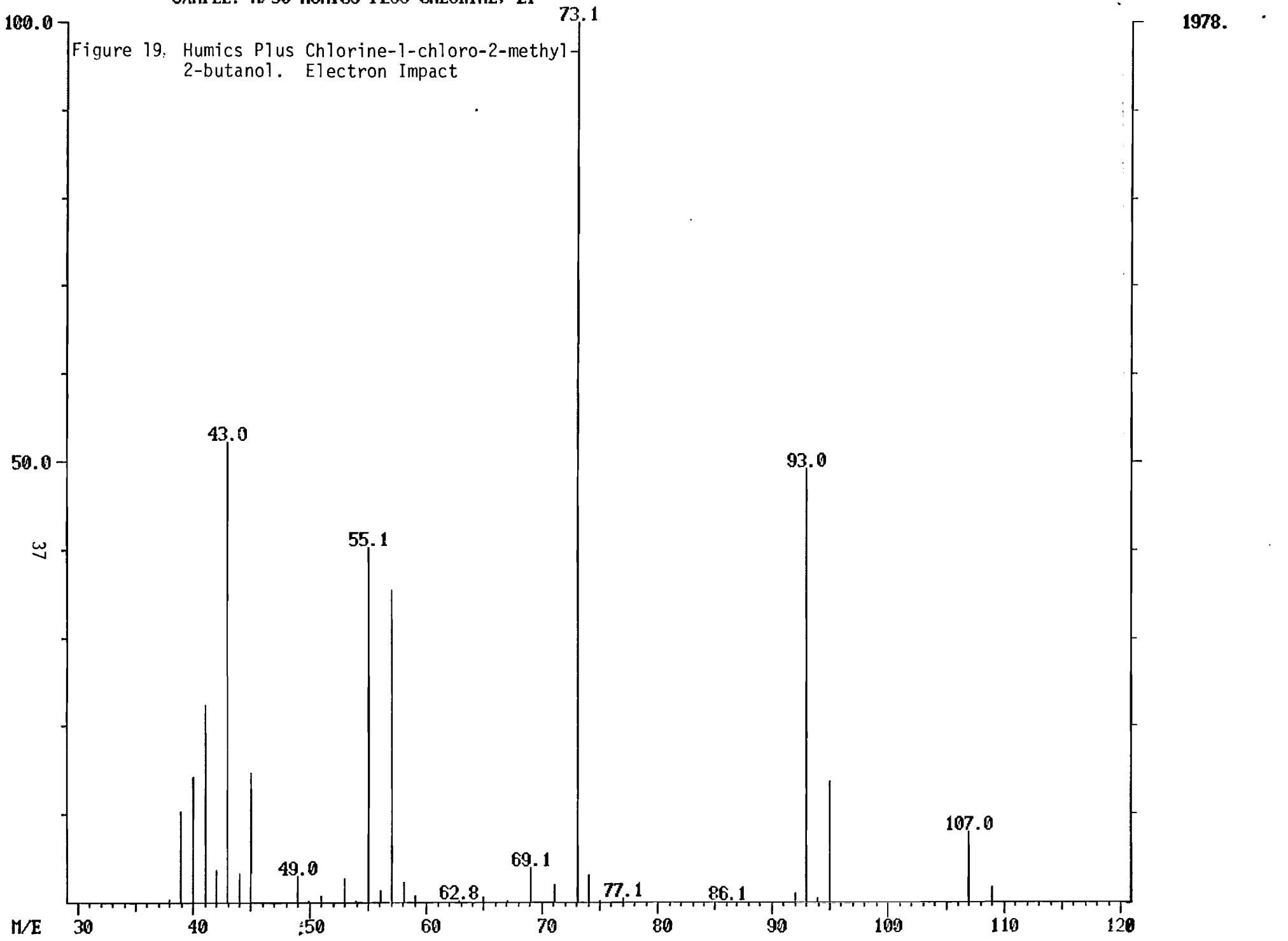
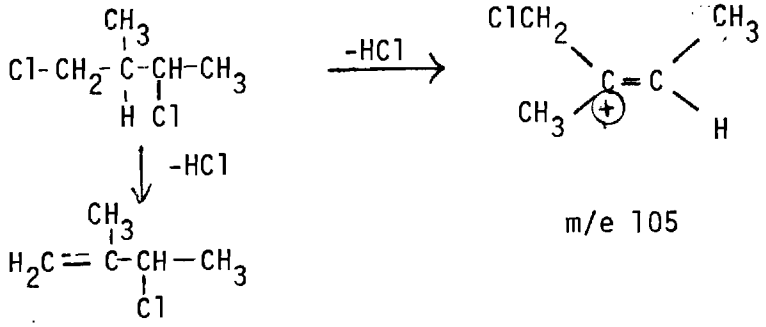


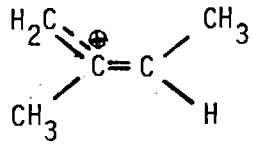
Figure 19. Humics Plus Chlorine-1-chloro-2-methyl-2-butanol. Electron Impact

G. This compound appears to be an isomer of dichloro-2-butane, probably 1, 3-dichloro-2-butane. The electron impact fragmentation pattern supports the 1, 3-substitution pattern. It will be recalled that the 2,3-dichloro structure identified as peak E lost Cl from the 2-carbon to form a tertiary carbonium ion of mass 105. In this case,, loss of HCl rather than just Cl allows formation of the more stable tertiary carbonium ion; loss of either chlorine is possible.



m/e 105

Either of these can lose a chlorine atom to form the ion at m/e 69.

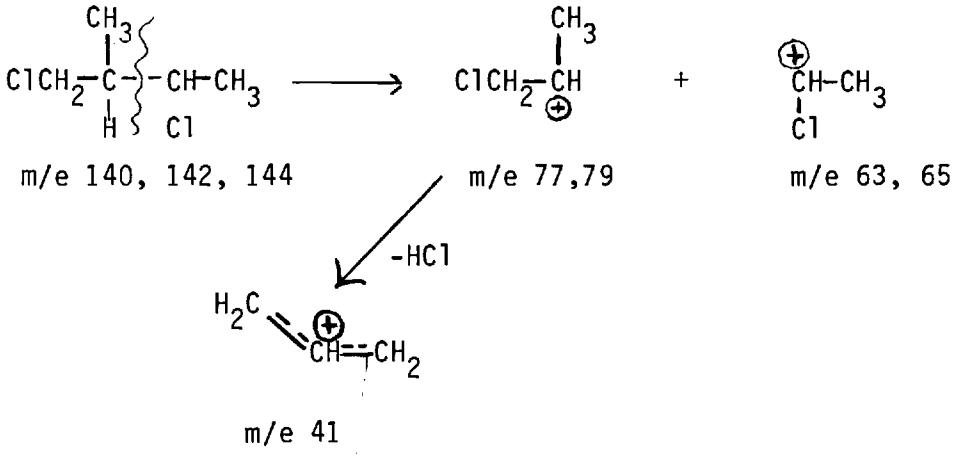


m/e 69

The ion of m/e 41 can be formed in several ways, including the loss of C_2H_4 from m/e 69,

m/e 69 m/e 41

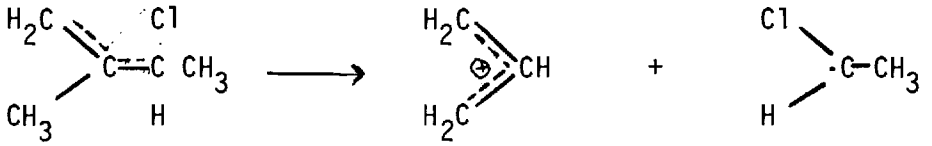
via mass 77, 79 (shown on nest page).



m/e 140, 142, 144 m/e 77,79 m/e 63, 65

m/e 41

or from one of the mass 104 structures,



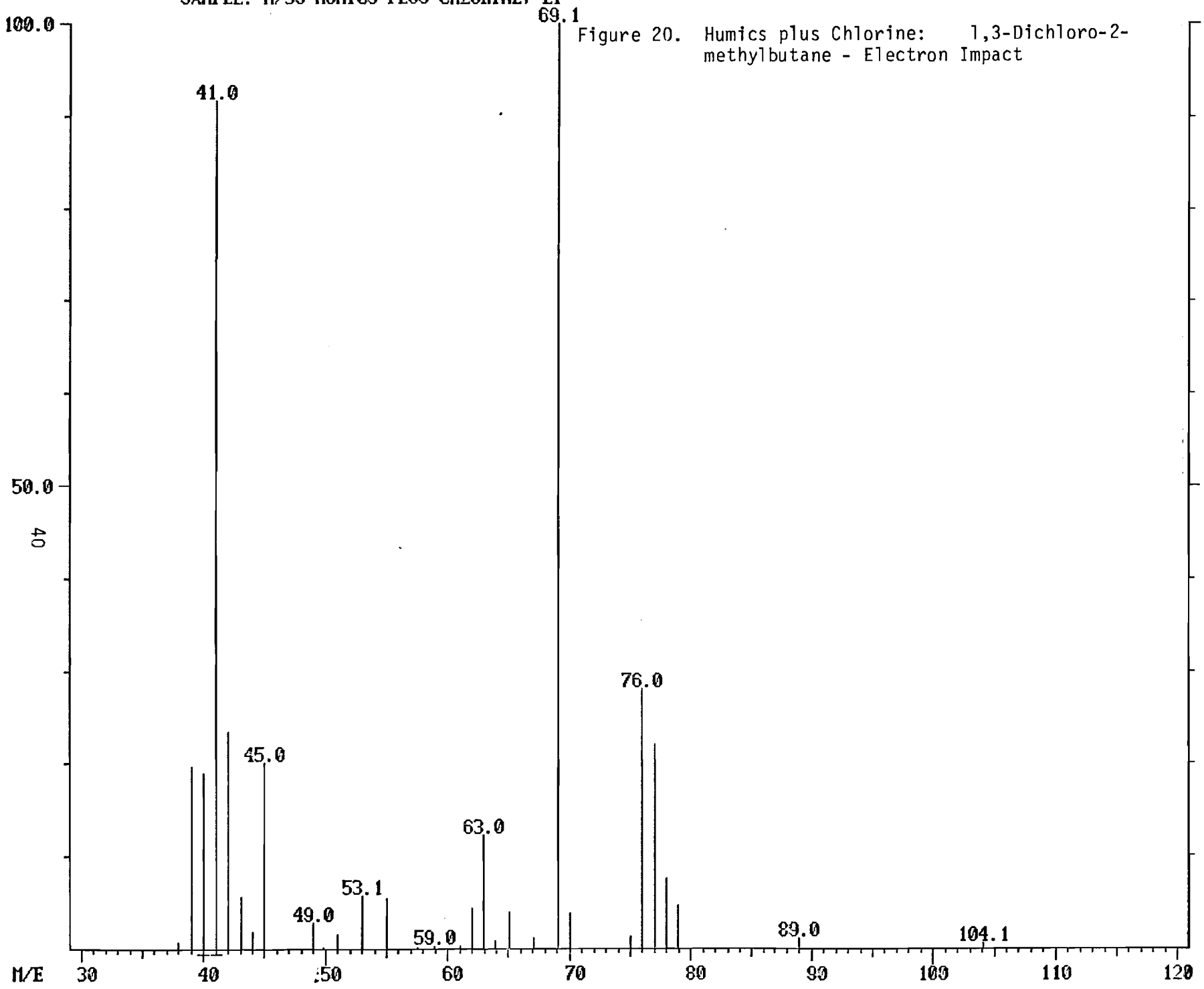
m/e 104 m/e 41 m/e 63, 65

Masses 49 and 51 arise from the terminal CH_2Cl group. Mass 45 should not appear in this mass spectrum, and it is in fact due to a shoulder peak, as shown by the mass chromatogram at that particular ion.

The chemical ionization spectrum shows essentially only three peaks, a m/e 105, 107 pair due to loss of HCl or C_2H_5Cl from MH^+ or $MC_2H_5^+$, respectively, and a small peak at 87 for which no explanation is readily apparent. The electron impact and chemical ionization fragmentation patterns are presented Figures 20 and 21 respectively.

05/08/78 15:29:00 + 15:31 CALI: 0597 #1 RIC: 6832.
SAMPLE: M/39 HUMICS PLUS CHLORINE, EI

Figure 20. Humics plus Chlorine: 1,3-Dichloro-2-methylbutane - Electron Impact



1754.

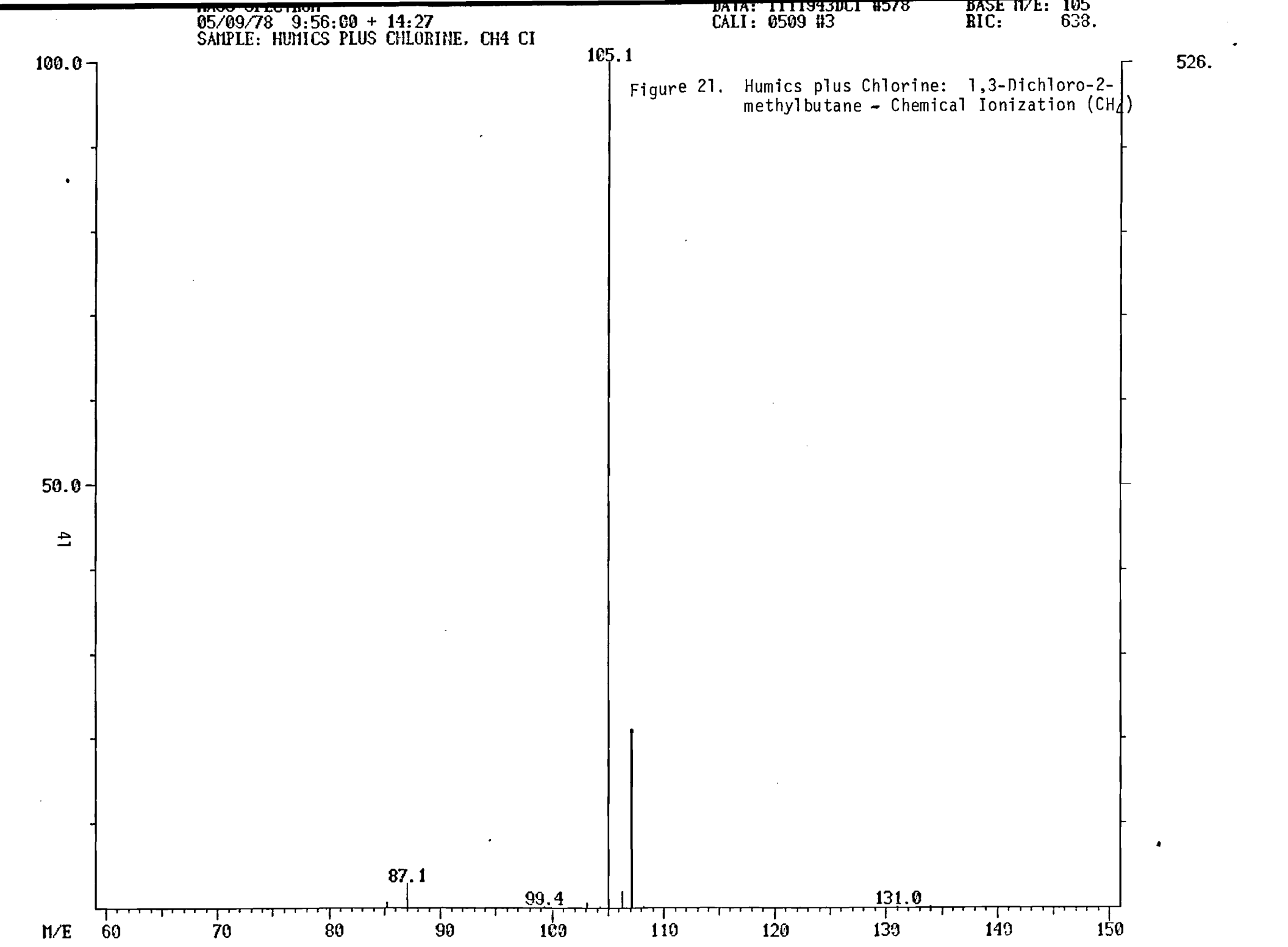
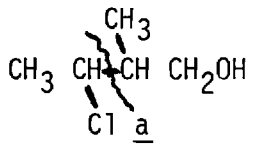


Figure 21. Humics plus Chlorine: 1,3-Dichloro-2-methylbutane - Chemical Ionization (CH_4)

H. 3-Chloro-2-methyl-1-butanol (or isomer) - It will be noted that this compound is isomeric with compounds C and F. Like C, its electron impact fragmentation pattern has m/e 59 as the base peak. Therefore, it is more similar to that structure than it is to F which has m/e 73 as its base peak. The suggested structure, would therefore seem to be the best fit - particularly since the loss of methyl is seen in the 2-butanol isomer which has two methyl groups adjacent to the carbon bearing the alcohol function while the proposed compound has none. Unfortunately we are not able to explain the ions at m/e 93 and m/e 95 which are more consistent with a structure related to F. Some fragment ions can be seen which explain many of the features of Figure 22.



m/e 122
(not seen)

Cleavage at a gives m/e 59 together with a very small amount of the chlorine-containing ion pair, at m/e 63 and m/e 65. The pair at m/e 93 and m/e 95 are derived from the parent ion by loss of C_2H_5. It is not clear how this happens since there is no ethyl group in the proposed structure. Loss of HCl from this ion would explain the strong peak at m/e 57. The lower mass ions are consistent with further fragmentation of the larger ions and have already been explained in earlier sections. It is possible that a better structure will be found. The chemical ionization spectrum which is presented in Figure 23 shows the loss of water from the quasi-molecular ion at m/e 105, 107 and the ion displacement product at m/e 87. Since the overall ion intensity was quite weak, the quasi-molecular ion itself was not evident in this particular spectrum. This ion has been observed, however, in more recent runs.

05/04/78 12:11:00 + 13:24 CAL: 0504 01 RIC: 5072.
SAMPLE: HUMICS PLUS CHLORINE, EI

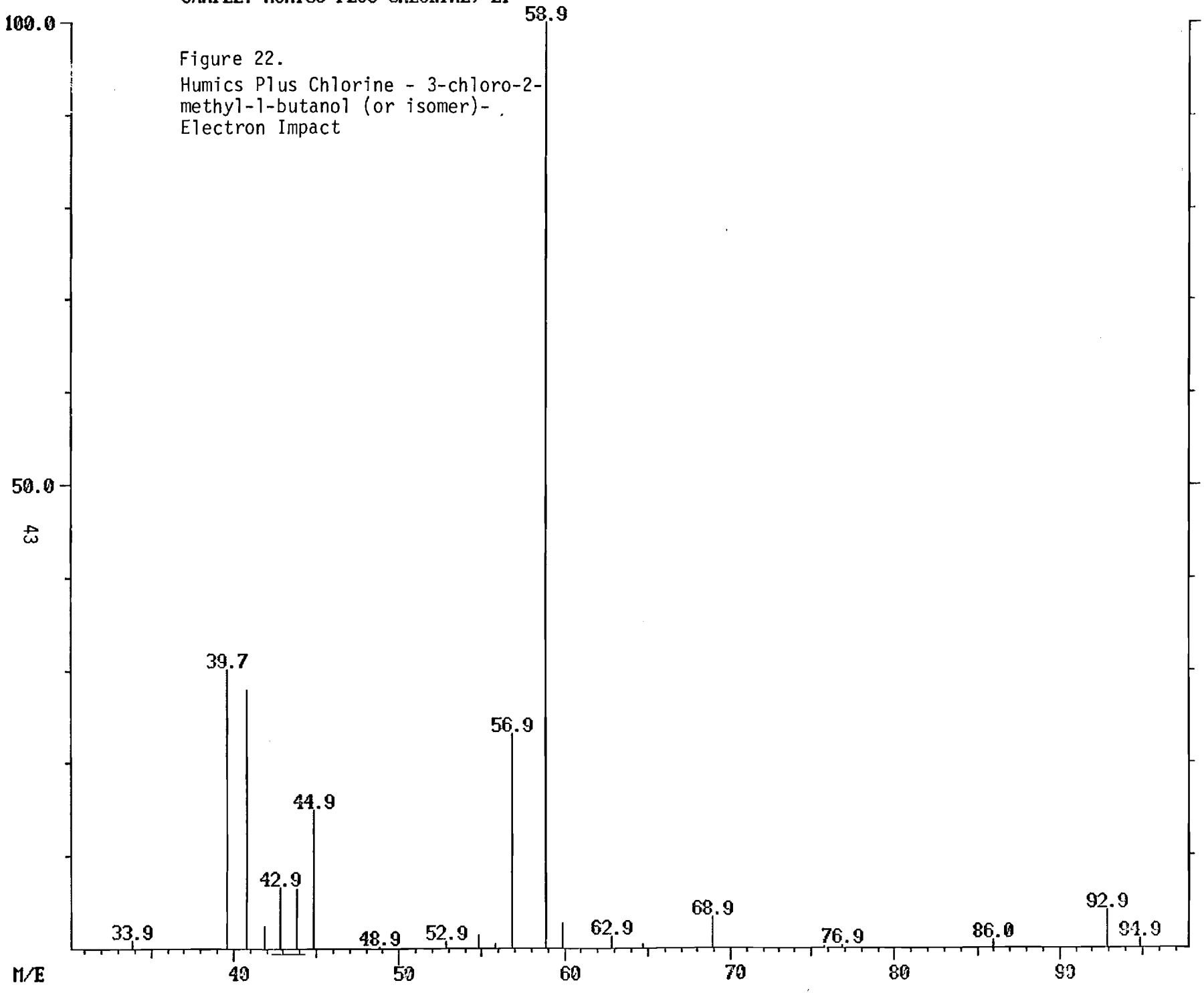


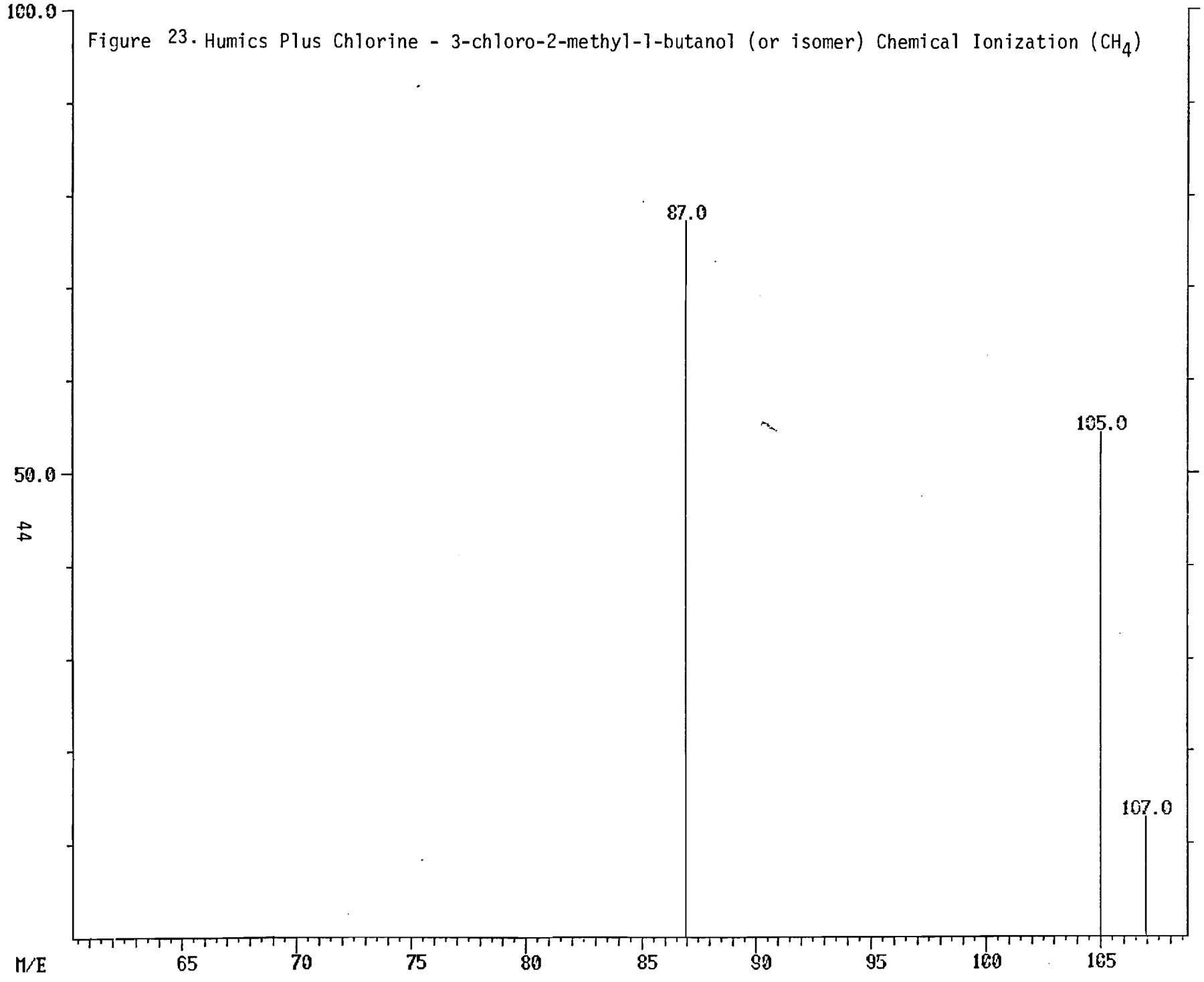
Figure 22.
Humics Plus Chlorine - 3-chloro-2-methyl-1-butanol (or isomer)-, Electron Impact

MASS SPECTRUM
05/04/78 14:57:00 + 15:34
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI

DATA: TTT1943CCI #623
CALI: 0504 #1

BASE M/E:
RIC: 173.

Figure 23. Humics Plus Chlorine - 3-chloro-2-methyl-1-butanol (or isomer) Chemical Ionization (CH_4)



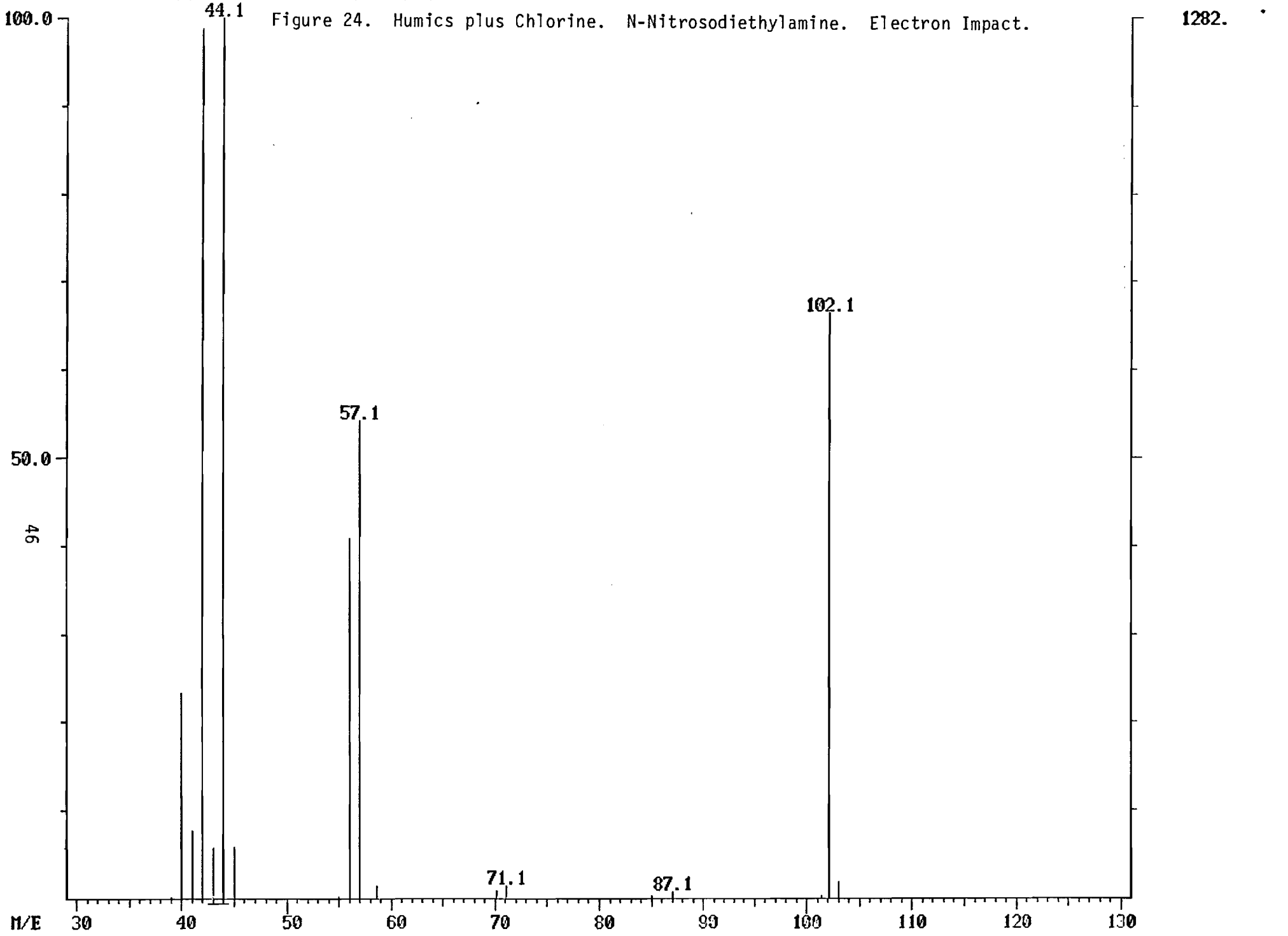
J. N-Nitrosodiethylamine. The electron impact mass fragmentation pattern (see Figure 24) of this gas chromatographic peak provided an excellent match with the library (fit indices all >959). The chemical ionization spectrum which is displayed in Figure 25 showed a strong quasi molecular ion together with the expected peaks at M + 29 and M + 41, thus confirming the molecular weight at m/e 102. The elctron impact spectrum also showed a strong molecular ion, weak losses of methyl and NOH at m/e 87 and 71 respectively together with strong ions at m/e 57 and m/e 56 corresponding to $C_3H_7N^+$ and $C_3H_6N^+$. The base peak at m/e 44 is most likely to be $N\text{-}N^+{=}O$ with a possible smaller contribution from C_2H_5NH. Simultaneous or rapid, stepwise loss of NOH and C_2H_5 could account for the strong ion at m/e 42. This ion might also be $CH_2{=}\overset{+}{N}{=}N$ since it is also very strong in dimethylnitrosamine.

This material is most likely an artifact introduced when adding the ethyl groups by reaction with N-ethylnitrosoguanidine.

K. Vigorous efforts are continuing to develop reasonable structures for all of the major peaks separable by capillary GC/MS. Our approach of starting with the solvent peak and proceeding to progressively longer retention times is a painstaking process and is probably producing fewer short-term results than might be obtained by working only on those spectra for which good matches can be obtained via the data system library. However, since the library cannot be expected to be a rich source of chlorinated natural products data, and since adjacent chromatographic peaks are likely to be structurally related, the thorough approach may bear more fruit in the long run. Certainly the isolation and structural elucidation of a series of halogenated isoprenoids is a significant result achieved using this approach. It is our intention to publish this work as soon as the documentation can be strengthened by results obtained from several other runs (some with flocculants have already been worked up for GC/MS analysis). We shall try to obtain authentic samples for final confirmation of structure. A full analysis of the control (which shows fewer

05/08/78 15:29:00 + 18:13 CALI: 0507 #1 RIC: 5248.
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI

Figure 24. Humics plus Chlorine. N-Nitrosodiethylamine. Electron Impact.



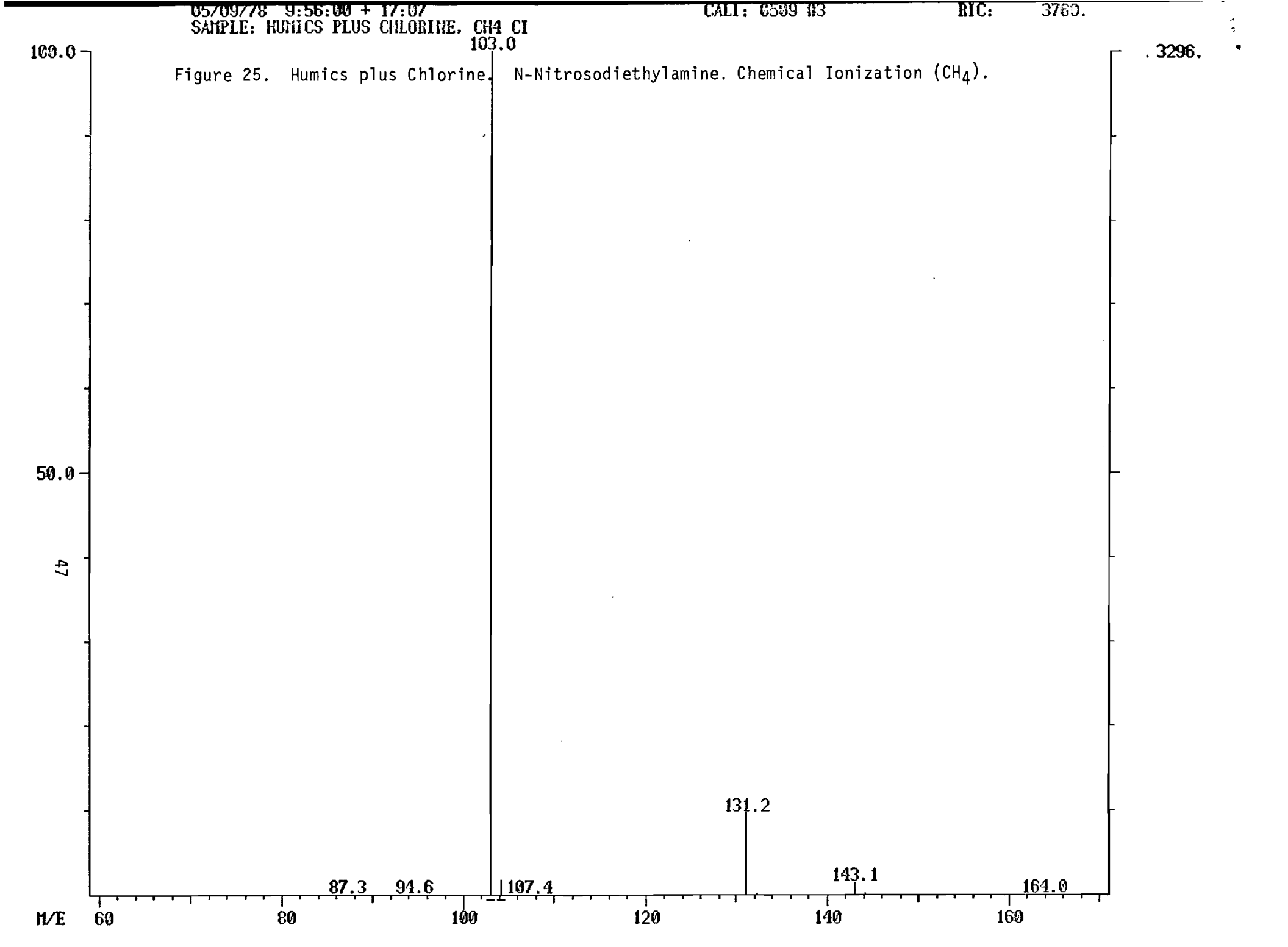


Figure 25. Humics plus Chlorine. N-Nitrosodiethylamine. Chemical Ionization (CH_4).

GC/MS peaks and no halogens) is next on the list of things to do. We would propose to continue to place primary emphasis on humic materials rather than on model compounds and trust that this is deemed by the sponsor to be performing within the proposed and agreed-upon scope of work. The use of other disinfectants (ClO_2) will proceed as soon as this work is completed. The sponsor may wish to suggest other disinfectants.

L. Quality Control

It should be pointed out that quality control is an important feature of this work. We frequently run test mixtures to test GC and data system performance and also check instrument performance with decafluoro triphenyl phosphine as recommended by the EPA protocol for priority pollutants. This has led to the discovery of a number of minor malfunctions which have all been corrected before they could lead to the production of erroneous data.

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

June 6, 1977

by

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68-01-4480

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DISCLAIMER

This report has been reviewed by the Criteria and Standards Division, Office of Water Supply, U.S. Environmental Protection Agency, and is intended for internal circulation only. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does the mention of trade names or commerical products constitute endorsement or recommendation for use.

I. PERSONNEL

The senior staff includes Dr. R. S. Ingols (retired, consultant), Dr. S. C. Havlicek, and Dr. J. H. Reuter. Other staff members include Dr. T. F. Craft, Mr. J. D. Lupton and Dr. S. Smith. Since their biosketches have already been provided as part of the original submission, it would seem more appropriate to describe in detail only those staff members who have joined the staff since the original submission.

A. Dr. I. El-Barbary - Dr. El-Barbary is a recent graduate of SUNY-Binghampton (Bruce McDuffie) who comes to us from the Environmental Health Administration in Washington, D.C. He is the author of eight publications in the area of environmental-analytical chemistry. His experience in analytical chemistry dates back to 1957 and includes 16 months at the Max Planck Institute fur Eisenforschung, studies on trace metals in the upper Susquehanna River Ecosystem, investigations on the sources and environmental fate of trace metals in the D.C. area and toxicological and bacteriological investigations of water quality in health care facilities. While his past experience is mostly with trace metal analysis, he has some knowledge of humic substances and is eager to make contributions on the organic side of environmental analytical chemistry. While he will be funded mainly out of this project (85-90%), it is anticipated that some use of his talents in the area of trace metal analysis will be made on another project. He will be employed as a postdoctoral research fellow.

B. Dr. M. Ghosal - This postdoctoral fellow is a graduate of Bihar University in India (1964) where he studied natural products chemistry.

His 19 publications date back to 1953 (masters work). In the early stages of his career, he had received several awards for academic excellence. He has extensive experience in the isolation and structural elucidation of natural products - including toxic substances and as such has "hands on" experience with LC, GC, IR, UV-VIS and NMR. He will be reporting directly to Dr. Reuter and will devote full-time service to this project.

C. Dr. J. W. Ralls - This worker brings some 25 years of experience to the project, having served most recently as Director of Research Services for the National Canner's Association. He has taken an early retirement to join our staff as a Senior Research Scientist and as such will replace Dr. Tsoukalas (who has recently resigned) on the project. He is the author of 43 publications and 14 patents. He has extensive experience in the isolation and identification of trace levels of organic compounds in a variety of matrices. He will devote about 60% of his time to this project.

D. Dr. L. W. Strattan - Dr. Strattan has been hired as a postdoctoral research fellow mainly on the basis of his "hands on" experience in mass spectrometry. He is a recent graduate of the University of Kansas (1974), has prior experience as a postdoctoral research fellow at Emory University and is the author of 11 publications - 19 of which deal with mass spectrometry. He will devote all or nearly all of his time to this project.

E. In addition, Ms. C. Livesay has been hired as a student co-op for developmental work in new areas. She has worked for two years in pesticide-related work at EPA's Research Traingle Park Facility and would be qualified

to step in on an as-needed basis should the situation demand it.

II. EQUIPMENT

As part of a major building program, the Energy and Environmental Analysis Division has recently established a Laboratory of Mass Spectral Analysis (LMSA) which is intended to handle analytical problems involving the techniques of mass spectral analysis. The major instrumental feature of this facility is the Finnigan Model 4023 Automated Switchable CI/EI GC/MS System. Some of the capabilities of this system are:

1) subambient temperature programming gas chromatography
2) switchable chemical ionization/electron impact ionization source
3) all-glass jet separator
4) tested performance to 1000 AMU
5) solid sample probe with subambient temperature programming
6) 32 K memory, 16-bit word central processing unit
7) dual density disc drive with 5.0 megaword storage
8) spectral library of more than 20,000 spectra
9) electrostatic printer/plotter
10) capillary column interface
11) ability to interact with remote libraries
12) INCOS automated system including state-of-the-art software and diagnostics
13) selected ion monitoring.

The Finnigan system is complemented by a Bainbridge-Jordan double focussing instrument which is used for problems requiring higher resolution but not requiring an interface with a gas chromatograph. For example, this

instrument has been used to distinguish between CO at m/e 27.9949, N_2 at m/e 28.0061 and C_2H_4 at m/e 28.0312. This system will shortly be interfaced with the INCOS automated data system to further expand our data acquisition/ processing capabilities.

In addition, the Laboratory of Mass Spectral Analysis is also equipped with several other magnetic sector instruments awaiting modification for specialized tasks, several gas chromatographs, and a liquid chromatograph. Further support equipment is available within EES for use should a need arise.

A Bellar and Lichtenberg purge-and-trap apparatus is being fabricated for the analysis of volatile organics in connection with this project. This route was selected in preference to the direct purchase of a commercial device partly on account of cost and partly because a greater flexibility in design is always possible with custom-made devices.

Due to a mis-processing of the equipment budget as materials and supplies by our department of contract administration, we find ourselves unable to order equipment items over $100. Unless a blanket correction is possible on the basis of your auditor's prior personal review of this part of the budget, we shall initiate formal correction procedures around the 15th of this month.

A variable wavelength detector for our LC is being evaluated. Of particular interest will be an investigation of the ability of this accessory to perform at the short wavelengths required for the analysis of saccharides.

III. GAS CHROMATOGRAPHIC STUDIES

The Becker, Tracor and Victoreen gas chromatograph were checked out and brought into operation during this reporting period. Parts were installed as required. All three instruments have been equipped with freshly packed, presilanized and conditioned GC columns and are ready to go.

In deference to the sponsor's expressed interest in some of the more biologically active substances which may be contained in the mixture of naturally occurring organic substances, some background work has been done on the gas chromatographic behavior of the trimethylsilylated (TMS) saccharides. A typical chromatogram of D(+) mannose is shown in Figure 1. The TMS derivatives were prepared using TRISIL-Z (Pierce) according to the manufacturer's instructions. The three peaks observed are all mannose with the smaller peaks representing minor anomeric forms. Some retention times are presented in Table I (conditions given on Figure 1).

Table I

Retention Times for Some TMS Sugars (In Minutes)

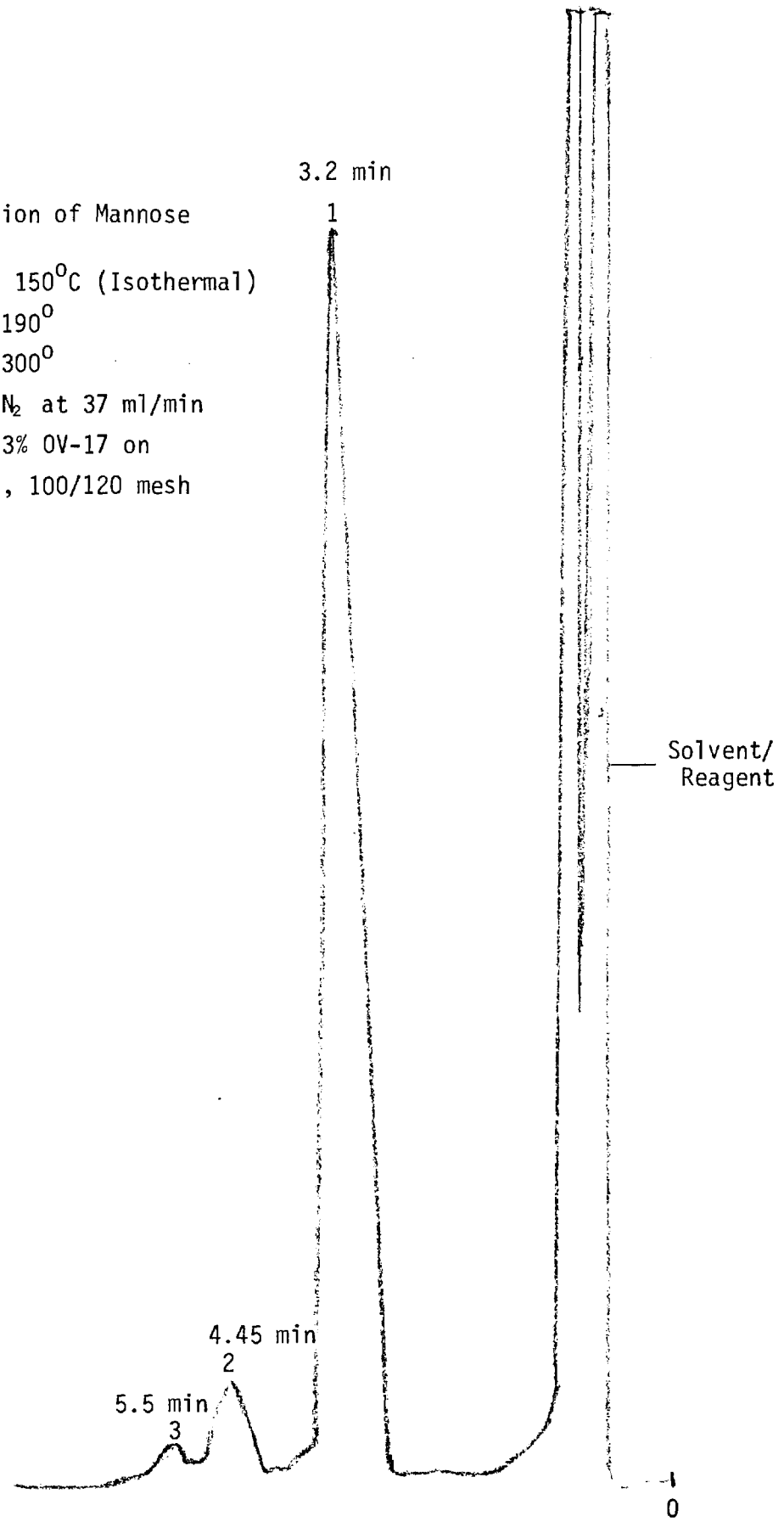
sugar	major peaks			minor peaks			
D(+) Mannose	3.2			4.45	5.5		
D(+) Glucose	8.75	4.6	6.9	5.2	5.8		
D(-) Arabinose	1.30			1.6	1.9		
D(+) Xylose	2.45			2.93	1.5	1.7	3.6
D(+) Galactose	5.2	4.0	3.3	6.4	8.0		

This work will be extended to include oliglosaccharides and amino acids in the near future.

Figure 1

TMS Derivation of Mannose

Temperature 150°C (Isothermal)
Injector: 190°
Detector: 300°
Carrier: N_2 at 37 ml/min
Column: 3% OV-17 on
Gas Chrom Q, 100/120 mesh



IV. SAMPLING AND PRELIMINARY SEPARATION

Samples of humic substances from the Satilla River are on hand. Since these samples were taken as a part of earlier work by Dr. Reuter, they have been subjected to freeze-drying without an effort having been made to first trap the volatile and semi-volatile fractions. Otherwise they are identical to samples which will be taken in the months which follow.

Humic substances from other origins will be used in sort of a "dry run" to test our technique and develop methodology without risking the more valuable river samples. Needed reagents and supplies, if not already on hand have been ordered. This work should be underway as of this reading.

V. PRELIMINARY CHLORINATION OF MODEL COMPOUNDS

An aqueous solution of phenol (11.4 mg/l) was prepared from chlorine-demand-free water. A portion of this solution (200 ml) was contacted at room temperature and neutral pH with enough of a concentrated chlorine solution to correspond to two moles of Cl_2 per mole of phenol. The overall chlorine concentration corresponded roughly to what might be encountered in normal water treatment procedures. All of the chlorine was consumed in about 15 minutes. A 250 ml aliquot was set aside for future analysis along with a sample of the chlorine-demand-free water.

A similar study was carried out with resorcinol. In this case, enough chlorine solution was added to correspond to three moles of chlorine per mole of resorcinol. Since the chlorine disappeared immediately, an additional 2 moles of chlorine was added. This, too, was gone after about 30 minutes. Aliquots were set aside as before.

Still another study was carried out with phloroglucinol. An amount of chlorine solution corresponding to six moles of chlorine per mole of phloroglucinol was contacted with the model compound as before. Only a trace of unreacted chlorine remained after 30 minutes. Aliquots were set aside as before.

A final study was performed with syringic acid which was brought into solution via the use of sodium hydroxide and then buffered to pH 7.1 with phosphate buffer before being treated with four moles of chlorine per mole of model compound. Chlorine uptake was slower with 1 mole disappearing during the first 15 minutes and 1/3 remaining even after two hours. It is interesting to note that a pinkish color develops after the residual chlorine is titrated.

This work is being undertaken to serve as a common reference point with Dr. J. J. Rook's recent report in Environmental Science and Technology (vol. 11, p. 478, 1977) so that a basis for comparison can be established between his work and ours.

VI. WORK WITH OTHER OXIDANTS

A chlorine dioxide generator has been designed by Dr. Ingols and will be assembled and tested shortly.

Work performed by Dr. Ingols in connection with another project has demonstarted that all commercial ozone generators do not necessarily generate ozone. this conclusion which would certainly surprise anyone not intimately connected with the field is based on the evidence shown in Table II.

Table II

Properties of "Ozone"

	From High Voltage Arc	From Ultraviolet (mercury)
I^- to I_2 (neutral)	+	+
M_n^{++} to M_n^{+7} at pH 1.0	+	-
Fluorescence with ethylene	+	+
Adsorbance in solution at 254 um	+	-
Formation of purple solid at liquid N_2 temp.	+	-
Oxidizing capacity increased by	dry gas	wet gas
Output gas	e^- deficient	e^- rich

These results indicate that the several ways of generating ozone: high voltage arc, corona discharge, UV at the mercury line and UV at 160 nm will have to be investigated.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report
July 8, 1977

by

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68-01-4480

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I. PERSONNEL

No personnel changes have taken place during this reporting period. A time shift of 10-20% from Dr. Havlicek to Dr. Ralls is contemplated so that better advantage might be taken of his extensive experience in this area. This change will have the effect of allowing the Principal Investigator to spread general laboratory management costs over our several on-going projects instead of charging them more heavily to this particular project.

II. EQUIPMENT

Delivery of the new GC/MS system is expected in early August. The Laboratory of Mass Spectral Analysis is being refurbished to accommodate this piece of equipment. A certain amount of new equipment will shortly be ordered using new developmental funds recently set aside from the FY 1977-78 budget. These items should further increase our ability to carry out this program. The exact nature of these purchases will be revealed as the extent of funding is more clearly established.

III. GAS CHROMATOGRAPHIC STUDIES

Samples of dilute solutions of phenolic compounds previously subjected to chlorination were analyzed for their chloroform content; the preparation of these samples was described in Section V of the preceding Monthly Progress Report. Additional work at higher pH's is described in Section V of this report.

One hundred ml portions of the five chlorination solutions and an equal volume of the water used to prepare the aforementioned solutions were extracted with reagent grade mixed hexanes (pentane would have been a better choice for an extraction solvent, but the required supply had not yet been delivered at the time the extractions were made). One 30 ml and two 10 ml

portions of mixed hexanes were used to extract the six samples. The combined extracts were dried over granular anhydrous sodium sulfate, and filtered without concentration, for injection into a GC unit.

The gas chromatographic unit used for the determination of chloroform was designed and constructed at EES/Georgia Tech for development of an improved electron capture (EC) detector by Mr. Joseph D. Lupton (see U.S. Patent No. 3,828,184 (1974)). The most recent modification of the basic detector design was used in the GC unit. A freshly packed 2 meters by 4 mm (i.d.) glass spiral column containing Poropak Q (80-100 mesh) was conditioned in nitrogen at 180^{o} for sixteen hours prior to use. Standards prepared from reagent grade chloroform in mixed hexanes were used to establish operating conditions. It was found that chloroform had a retention time of 8.25 minutes at a column temperature of 152^{o}. The nitrogen carrier gas was operated at 20 psig and the estimated flow (using mass flow transducer (Model LF5K) and mass flow meter, Matheson Gas Products, East Rutherford, N.J.) was 20 ml/min. The mixed hexane components appeared to have very long retention times under these conditions. (Earlier experiments with an OV-1 column operated at 25^{o} did show response to mixed hexane components with the EC detector at short retention times. The Poropak Q column was operationally useful for determination of chloroform in mixed hexanes since for the first six hours of operation, after conditioning for 12-16 hours, only a detector response to chloroform was observed. After multiple injections over a six hour period, increase of baseline drift rates and broad peaks were recorded, apparently from the slow elution of hydrocarbon compounds from the mixed hexanes.

A set of chloroform determinations were obtained using a one micro-liter, pressure-tight, syringe. The precision of response to multiple injections of chloroform standards, made over a period of four hours was quite good, as shown by the tabulation in Table I.

Table I

Electron Capture Detector Response to
Repetitive Injections of a Chloroform Standard

Run Number		Recorded Response mm^2/ng
I-15-1		4.06
I-15-2		4.30
I-15-3		4.52
I-15-4		4.20
	Mean	4.27
	Standard Deviation	0.18
	Standard Error	±0.09

The results for the measurement of chloroform in six extracts are presented in Table II.

Table II

Chloroform Concentration of Chlorinated
Aqueous Solutions of Phenolic Compounds

Sample Number	Compound Added	pH	Hexane Extract ng/µl	Aqueous Solution µg/100 ml
I-7-B	None	7	0.09	4.5
I-7-B	None	7	0.07	3.5
I-7-1	Phloroglucinol	7	0.18	9.0
I-7-2	Resorcinol	7	0.39	20
I-7-3	Phenol	7	0.05	2.5
I-7-4	Resorcinol	10.2	0.61	30
I-7-5	Phloroglucinol	10.3	0.06	3.0
I-7-6	Syringic Acid	7	0.07	3.5

The ultraviolet spectra of the chlorinated phenolic compound solutions showed weak general absorption at 190-360 nanometers for the solutions containing resorcinol and phloroglucinol and more specific absorption maxima for the phenol and syringic acid solutions. These results indicated less complex reactions for the latter two phenolic compounds.

Three conclusions may be drawn from the results of this study of chlorination of dilute aqueous solutions of phenolic compounds:

1) The tap water from the City of Atlanta water supply contains Chloroform in a range of 27-42 ppb. This result should be compared with the value of 36 ppb found in the 1975 EPA survey of municipal drinking waters.

2) Chlorination of water containing resorcinol and phloroglucinol results in the formation of chloroform and confirms the report of Rook (Rook, J.J., J. Water Treatment Examination, 23, 234 (1974); ibid., Environ. Sci. and Tech. 11(5), 478 (1977)).

3) Chlorination of phenol and syringic acid produces unidentified intermediate compounds which do not lead to chloroform as an end product. The uptake of chlorine by these solutions suggests that changes are taking place. The nature of these changes will be better defined in future studies.

Considerable progress was made in determination of phenolic compounds as their trimethylsilyl ethers (TMS) in preparation for analysis of this class of organic compounds in drinking water supplies. The TMS derivatives were prepared using TRISIC-Z® reagent. The GC conditions used were: 2 meter by 4 mm (i.d.) silinized stainless steel column packed with 3% OV-17 on 100-120 Gas Chrom Q support (conditioned in nitrogen at 210° for six hours); Nitrogen carrier gas flow rate was 9 ml/min.; injection port was 190° and flame ionization detector was operated at 290°. The following temperature program was found to bring about good separations: 100° for 3 min., an increase of 7.5°/min. to 210° and a 2 min. hold at 210°. It was essential to cool the column oven to about 60° after each run to get temperature at start of program at 100° in order to separate the TMS-phenol from the solvent peak. Using these conditions, four representative TMS

derivative of phenols were clearly separated. The retention times for these compounds are recorded in Table III. A copy of a typical run is shown in Figure 1.

Table III

Retention Times of Trimethylsilyl Ethers of Phenolic Compounds

Parent Phenol	Retention Time (min.)
Phenol	1.2
Resorcinol	6.1
Phloroglucinol	10.9
Syringic Acid	15.5

A literature search was made and reagents were ordered to prepare and separate trifluoroacetylbutyrate derivarives of protein-derived α-amino acids.

IV. SAMPLES AND PRELIMINARY SEPARATION

In preparation for characterization of fractionated humic compounds from Satilla River water samples, the method of Brooks, et al., (Austr. J. Applied Sci. 8, 206 (1957)) for total hydroxyl group determination has been tested. A modification of the method in which a humic sample is acetylated with pyridine-acetic anhydride, water is added to hydrolyze the excess anhydride, and the resulting highly colored solution is titrated with standardized sodium hydroxide solution to an end-point determined with a pH meter. This method appears promising. Control runs with no humic substances present gave low values for total hydroxyl and a series of results from humic samples gave good precision for total hydroxyl values.

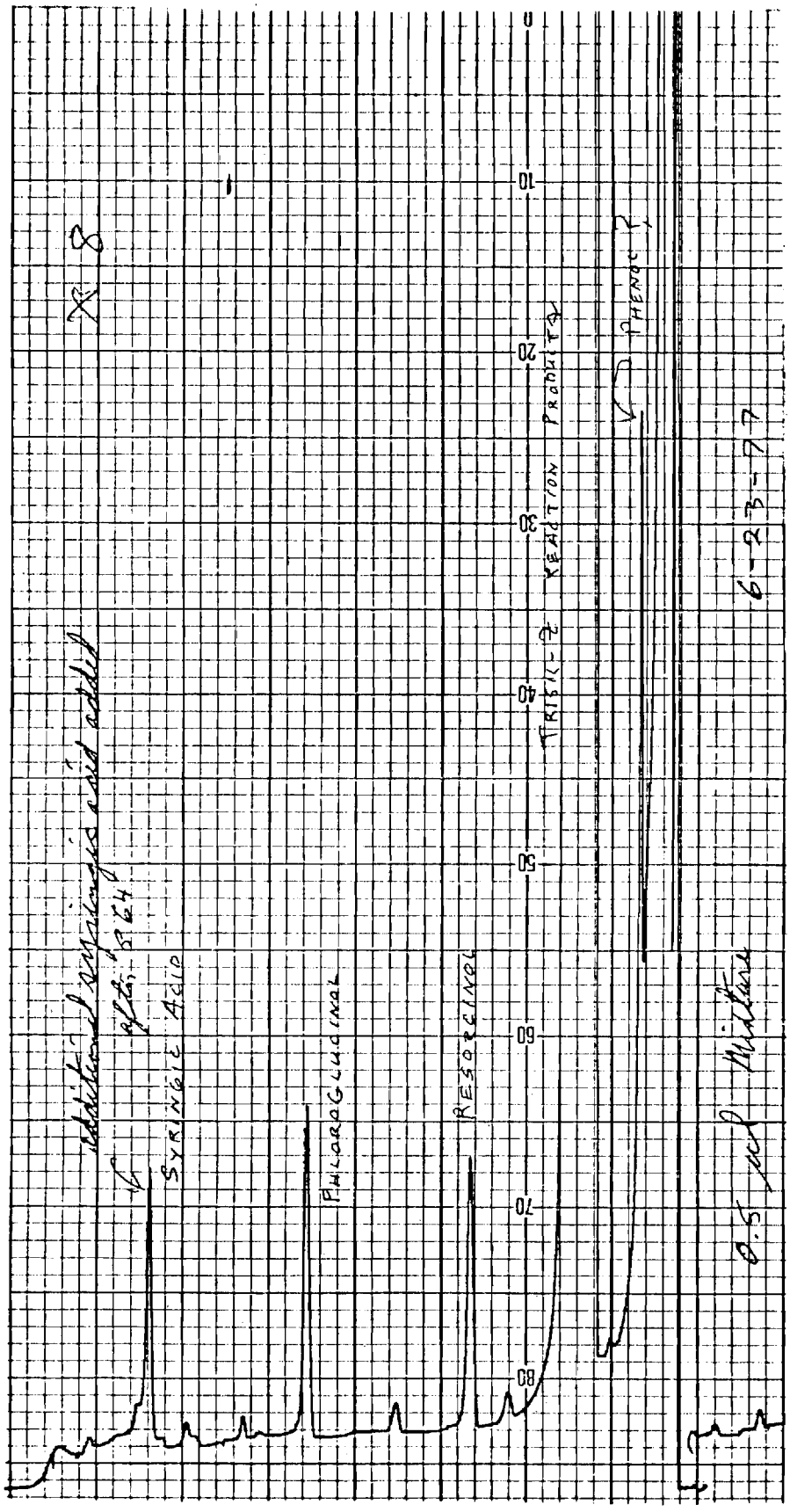


Figure 1. Separation of trimethylsilyl ethers of phenolic compounds.

V. PRELIMINARY CHLORINATION OF MODEL COMPOUNDS

A solution containing 13.4 mg/l of phloroglucinol was adjusted to pH 10.3 by addition of aqueous sodium bicarbonate and sodium hydroxide. Treatment of the resulting solution with six equivalents of chlorine produced color development followed by a slow fading. The chlorine residual persisted for several hours before reaching a non-detectable level. A solution of 8 mg/l of resorcinol at pH 10.2 was treated with six equivalents of chlorine at room temperature. The purple color which formed at first rapidly faded to a yellow. After three hours, there was a just detectable chlorine residual level.

VI. PRELIMINARY STUDIES ON GENERATION OF DIAZOETHANE

It was found that the rates of stirring of the reaction mixture of aqueous-alcoholic potassium hydroxide solution and ethereal 1-ethyl-1-nitrosourea was critical for diazoethane formation. The yield of diazoethane was determined by titration of unused excess citric acid with standard sodium hydroxide after the ethylation of citric acid by the diazoethane had been completed. The first two runs gave much lower yields than the 50-70% conversions reported.

VII. OZONE STUDIES

An attempt was made to generate singlet oxygen from hypochlorite and hydrogen peroxide according to the method of Foote and Wexler (J. Am. Chem. Soc., 86, 3879 (1964)). It was anticipated that the product could then be compared with photochemically generated "ozone" in order to gain further insight as to the nature of the photo-oxidants. Unfortunately, the success of this chemical generation is uncertain at this time since the gas production which was observed did not produce a corresponding change in the oxidation-

reduction potential or the pH. Since the cited reference is only a communication, it may be that some essential detail has been omitted or it may be that the measurement technique is not appropriate for what may be a very transient intermediate. The reaction was attempted at a number of different pH values—none of which provided any evidence that an oxidant which differed from the initial materials was present.

Preliminary observations of oxidation-reduction potentials in water vs. pH for photochemically generated ozone showed a slight increase in values over those obtained for air alone. This difference was inversely related to pH.

A more detailed study of the difference in the reactivity towards manganous ion of ozone from a high voltage arc and that produced by ultraviolet light is outlined below in tabular form. It will be noted that even the photochemical oxidant can produce small amounts of permanganate at pH 7.0.

Properties of "Ozone" vs. pH

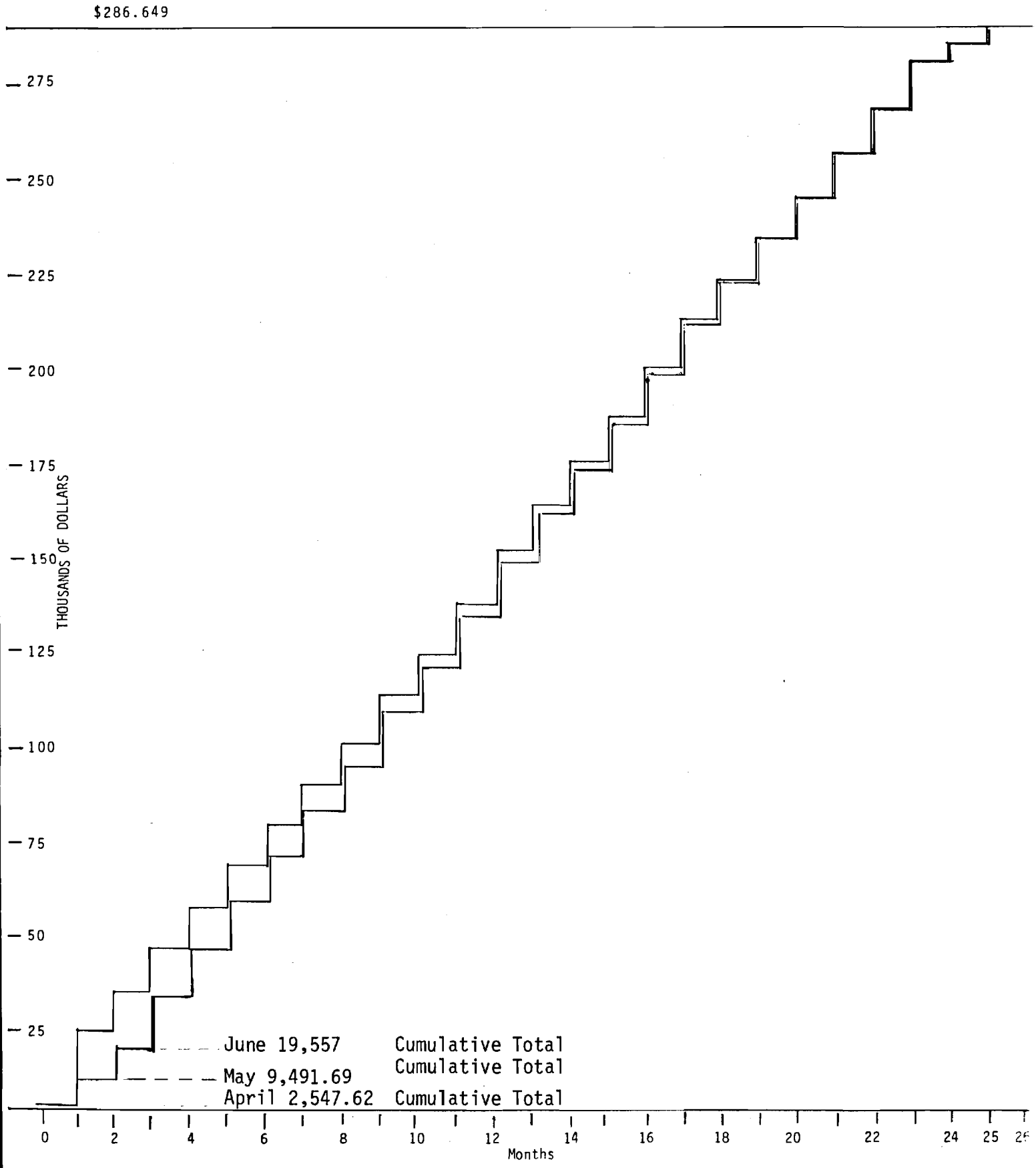
pH	High Voltage Arc	Ultraviolet
1.0	Mn^{+7}	Mn^{+4}
2.2	Mn^{+7}	Mn^{+4}
7.0	Mn^{+4}	Mn^{+7}
9.7	---	Mn^{+4}
12.0	---	Mn^{+4}

In a separate experiment, resorcinol was treated with photochemical ozone at a concentration of 20 mg/l (pH 7.0, room temperature) for one hour. After this period of time, unreacted resorcinol could not be detected in the UV spectrophotometer. A parallel reaction with ozone from the high voltage arc will be conducted during the next reporting period. Product characterization is in progress.

A number of articles by Dr. Jürg Hoigné have been provided by our colleague Rip Rice of Jacobs Engineering Company and appear to be in agreement with our observations that much "ozone" chemistry does not proceed from ozone at all. These articles are being translated by the staff on a time-available basis.

FINANCIAL MANAGEMENT REPORT - June

GEORGIA TECH RESEARCH INSTITUTE - CONTRACT No. 68-01-4480



E-20-657

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August 5, 1977

Dr. Joseph Cotruvo
Environmental Protection Agency
401 M Street
Office of Water Supply (WH-550)
Criteria and Standards Division
WSME, Room 1030
Washington, DC 20460

Dear Dr. Cotruvo:

Enclosed is our progress report for the month of July. I appreciate your having granted us permission to turn in our graph Financial reports on the 15th of the month. This will save time and minimize the chance for error as we can use our internal Financial reports which I get on the 12th of the month as the basis for the report which you will receive.

We have had some rain recently and just a little bit more should put enough water back in the Satilla River so that we can get some good samples. I trust you will find this report satisfactory.

Sincerely,

S. C. Havlicek, Ph.D.
Project Director

SCH/ml

DISCLAIMER

This report has been reviewed by the Criteria and Standards Division, Office of Water Supply, U.S. Environmental Protection Agency, and is intended for internal circulation only. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

I. PERSONNEL

No personnel changes have taken place during this reporting period.

II. EQUIPMENT

Engineering surveys to guarantee the availability of adequate air-conditioning for the new GC/MS system have been made. Current air-conditioning capacity is more than adequate. Minor modifications of existing power supplies will be required. This work is scheduled to be completed by the time the instrument arrives. In order to speed work on this and other projects, Georgia Tech has ordered an analytical balance to be placed directly within the laboratory, thus eliminating the need for time-consuming trips to adjacent facilities. A new Pye Unicam SP 1000 infrared spectrophotometer is now in place in our laboratory as is a new Perkin-Elmer LC 55 variable wavelength UV-VIS detector for our LC. A reflecting beam condenser and microcell assembly have been ordered for the infrared spectrophotometer so that sub-milligram samples can be examined. All of these general purpose items have been purchased out of Station funds and are mentioned only in the context that they will have a favorable impact on the sponsor's research program.

An attenuator stand and scanning accessory have been ordered for the liquid chromatograph. These two accessories were charged to project funds as part of the agreed-upon LC equipment update. This equipment will permit us to obtain a complete UV-VIS spectrum from the LC fractions as they are eluted from the instrument. Hopefully this will enable us to obtain a more complete characterization of complex organic mixtures such as will be encountered in carrying out this research.

A Finnigan Model 9500-6C gas chromatograph equipped with the Lupton porous anode EC type detector has been brought to operational status and

will be used to develop conditions for resolution and identification of compounds isolated from compound reactions. Preliminary debugging work has shown no objectionable drift between 150°C and 210°C during temperature programming at both 10°C/min and 20°C/min. It is anticipated that this range can be substantially expanded. Injections of up to 8 µl of 100% chloroform show a complete return to baseline in less than 2 minutes. This rapid recovery of the detector is seen in Figure 1. Detector sensitivity, of course, is 300 times less than under normal operating conditions.

Three types of light induced "ozone" generators are on hand. This equipment is on loan from commercial manufacturers. The loan of a corona discharge-type ozone generator has been promised. This piece of equipment should arrive in about two weeks.

III. GAS CHROMATOGRAPHIC STUDIES

The methodology for determination of trace levels of naturally occurring amino acids has been investigated in some detail. The review by P. Husek and K. Malek[1] indicates that the Gehrke method[2] using butyl trifluoroacetate derivatives of amino acids has been widely used in applications studies. Therefore, we have selected this method for use in connection with our studies.

After treatment of the amino acids with butanol-hydrochloric acid and trifluoroacetic anhydride, the resulting derivatives were subjected to gas chromatographic separation both individually and as mixtures. Figure 2 shows the separation of a mixture of 19 amino acid derivatives on a column containing 2% OV-17 and 1% OV-210. The retention times for gylcine, leucine, phenylalanine, tyrosine and glutamic acid were established by preparation and chromatography of derivatives of the individual amino acids. Ten-component and nine-component mixtures were prepared with the components

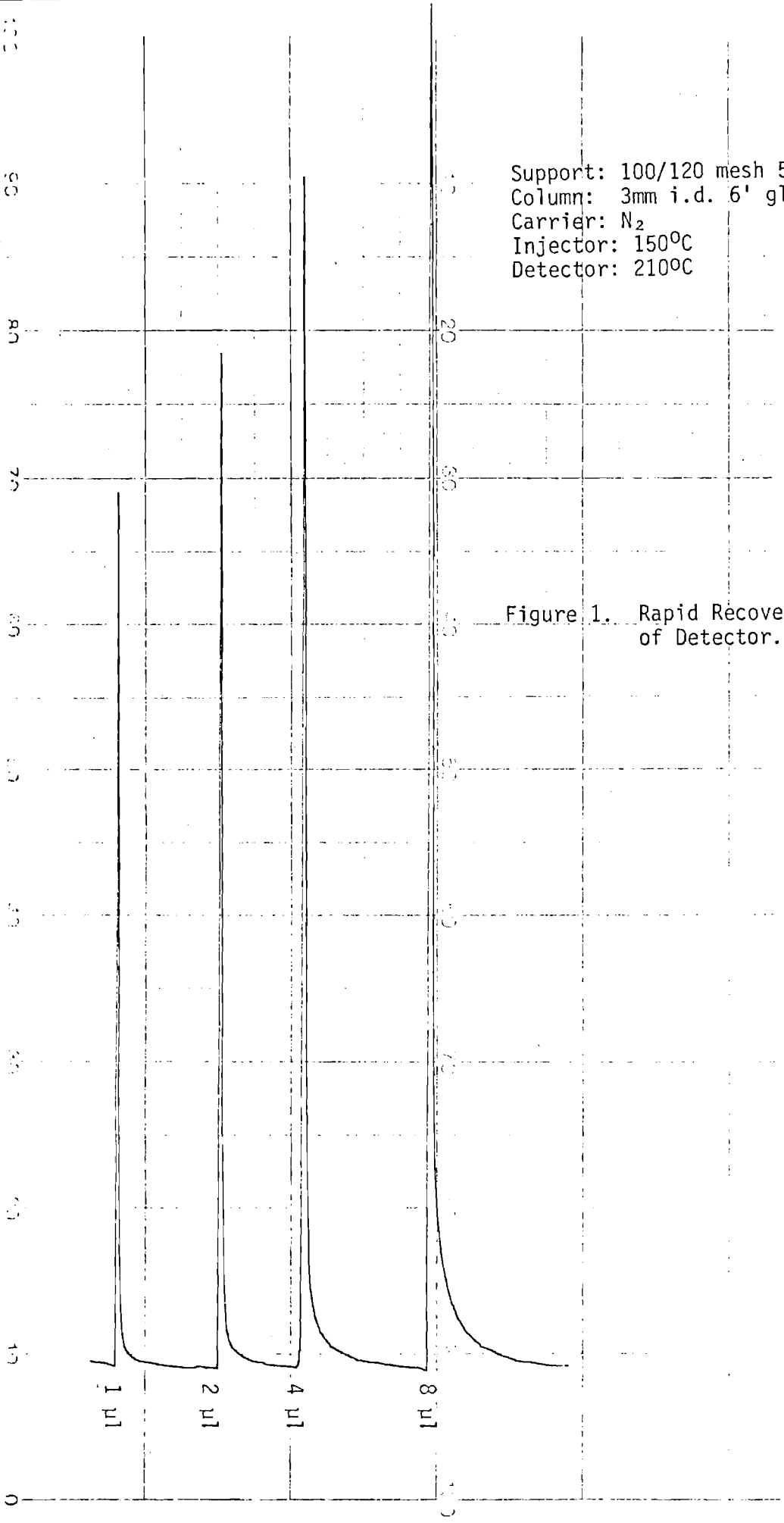


Figure 1. Rapid Recovery of Detector.

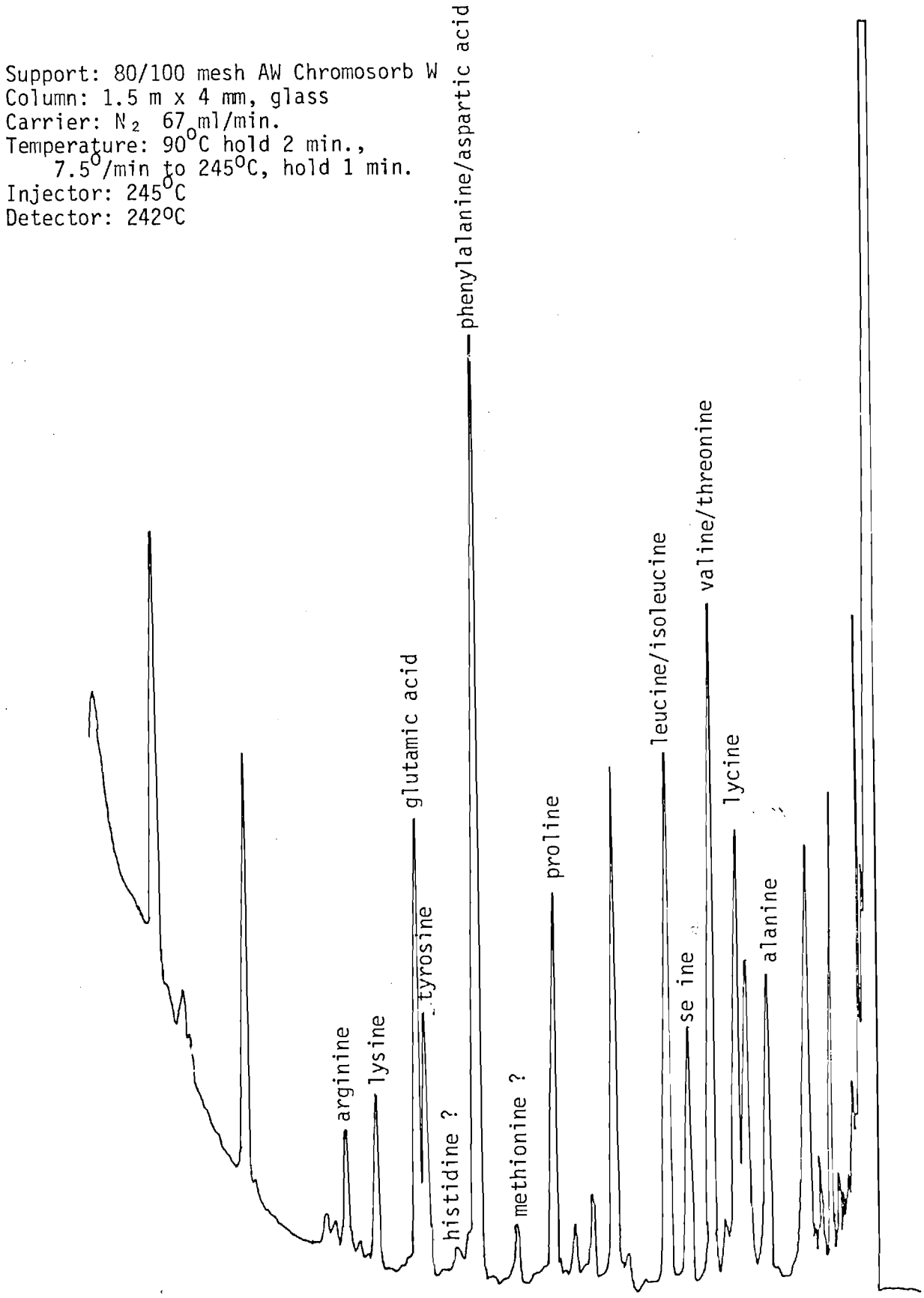


Figure 2. Separation of N-trifluoroacetyl-n-butyl ester Derivatives of Amino Acids on 2% OV-17/1% OV-210.

of each mixture selected in an alternate fashion on the basis of the published elution order for these derivatives. The correspondence of retention times observed with component identity for the two mixtures was partially established from results with the five individual derivatives and published elution orders.

The separation of the same nineteen amino acid derivatives on a column containing 0.6% ethylene glycol adipate (EGA) is shown in Figure 3. Although relative retention times from the EGA column chromatograms are uncertain at present, the components of the mixture are well separated. Identification by GC/MS should be straightforward.

Table I gives the retention times for the separation shown in Figure 2. The conditions used for this separation were: 1.5 m by 4 mm i.d. silinized glass column packed with 2% OV-17 and 1% OV-210 on 80/100 mesh, acid-washed, Chromosorb W, nitrogen carrier gas flow, 67 ml/min; column temperature was programmed from 90°C (2 min.), increase of 7.5°C/min to 245°C with 1 min. hold; injector temperature, 245°C; detector temperature, 242°C (FID).

IV. SAMPLES AND PRELIMINARY SEPARATION

A. Extraction of Aquatic Humics From River Water

The objective of the following experiments was to establish an efficient and chemically "safe" extraction procedure of aquatic humics from river water.

Both Amberlite XAD-7 and XAD-8 were extracted overnight in a Soxhlet extractor first with methanol, then with water. Resin beds (1 x 18 cm) in glass columns were prepared in water by filling the columns with a slurry and then backflushing to allow settling of resin beads according to size.

Aqueous solutions of humics (100 mg/l), acidified to pH 1.7 with HCl, were allowed to pass slowly through the resin beds. XAD-7 was found to be considerably more efficient in absorbing dissolved humics than XAD-8, although by choosing larger column diameters and smaller flow rates, XAD-8

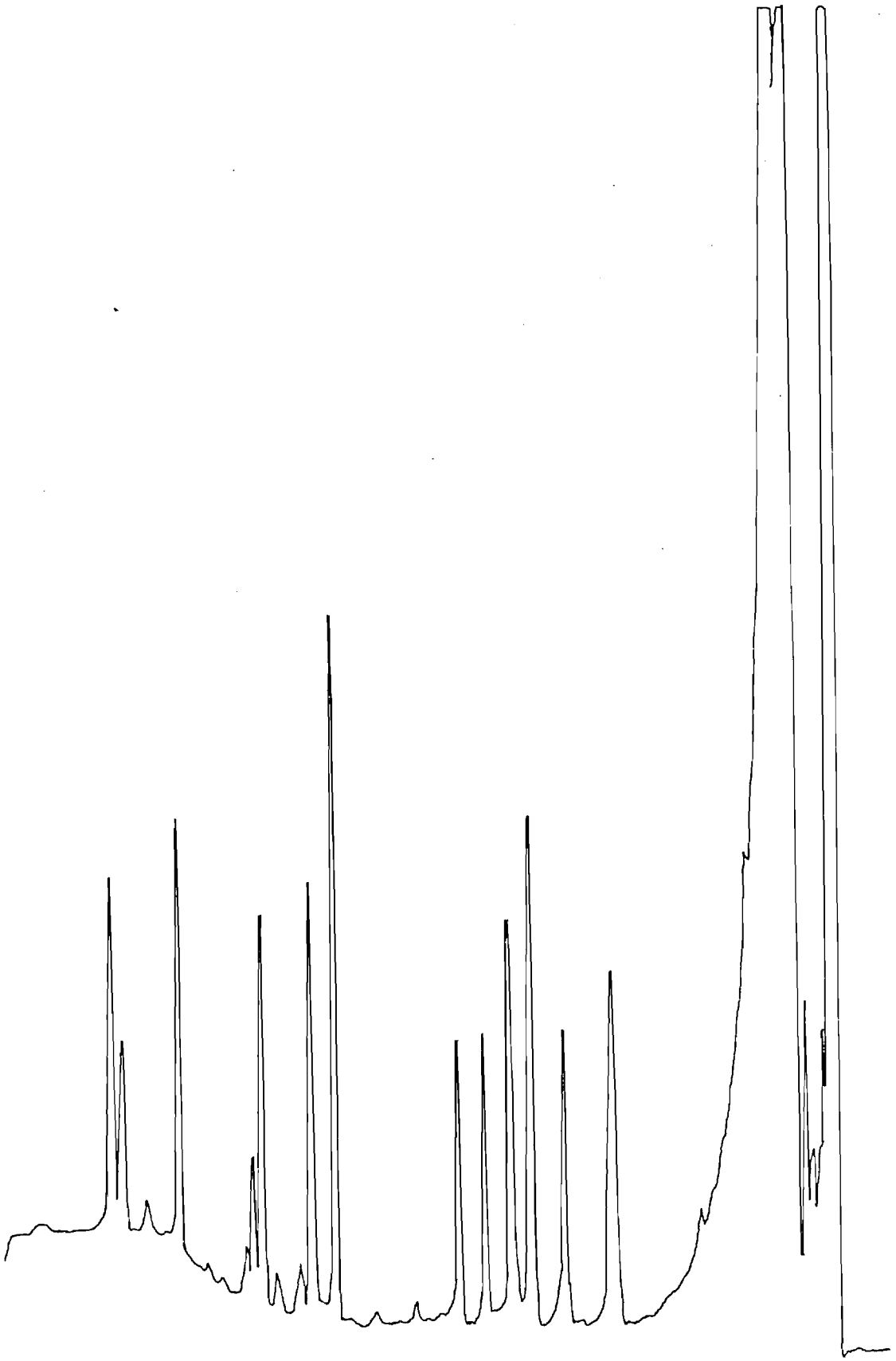


Figure 3. Separation of N-trifluoroacetyl-n-butyl ester Derivatives of Amino Acids on 0.60% Ethylene Glycol Adipate

Table I

RETENTION TIMES FOR N-BUTYL TRIFLUOROACETYL-DERIVATIVES OF AMINO ACIDS

Parent Amino Acids	Retention Time*	
	min.	relative to glutamic acid
Alanine	4.0	0.25
Glycine	4.8	0.30
Valine/Threonine	5.5	0.35
Serine	6.5	0.41
Leucine/Isoleucine	7.3	0.46
Proline	11.0	0.70
Methionine	12.5	0.79
Phenylalanine/Aspartic Acid	13.8	0.87
Histidine	14.5	0.92
Tyrosine	15.5	0.98
Glutamic Acid	15.8	1.00
Lysine	17.3	1.09
Arginine	18.3	1.16

*The retention times of cystine, cysteine and asparagine are uncertain at the present time.

proves to be sufficient absorbent. Better than 95% of the color was removed. Desorption of the humics from the XAD resins with dilute aqueous base lead to almost quantitative recovery. However, a choice of aqueous bases had to be made. Dilute NaOH (0.01N) requires desalting through acidic cation-exchange. It was found that this desalting step leads to high material losses (up to 30%) by irreversible absorption of the organic materials on the ion-exchange resin. This loss is unacceptable for our studies, especially since we found that the losses were not uniformly distributed over the whole range of molecular weights. The higher molecular weight fractions were absorbed more efficiently than were the lower molecular weight materials. The next choice for a suitable aqueous base could be dilute NH_4OH. However, at higher pH values (>8), phenols with at least two hydroxyl groups in o- or p- position react with ammonia to give dark colored nitrogenous polymers. Since we suspect that the humics under investigation contain such functional group orientations, and indeed may contain some nitrogenous materials of this type, we have been reluctant to exercise this choice. The chances of inducing structural alteration by the use of ammonia cannot be tolerated in the present study. Two of the hydrogen atoms of ammonia seem to participate in the polymerization reactions: dimethylamine reacts with p- benzoquinone to form bis-dimethylamino-quinone, whereas monomethylamine leads to resinous products. No high molecular weight nitrogen containing products, on the other hand, are formed with trimethylamine. We therefore have chosen trimethyl-amine as the desorbing base for aquatic humics absorbed on XAD-7 or XAD-8.

We want to point out that sampling of river water in South Georgia is at the present time inopportune. The rivers are at low base flow, so most of the water is derived from the water table and is very low in organic matter. This situation is obviously quite abnormal. We will have to wait until sufficient rainfall brings the water table close to the surface. We

expect normal or even increased loads of organic matter as soon as the abnormal drought conditions are over.

B. Oxidative Degradation Studies on Aquatic Humic Matter

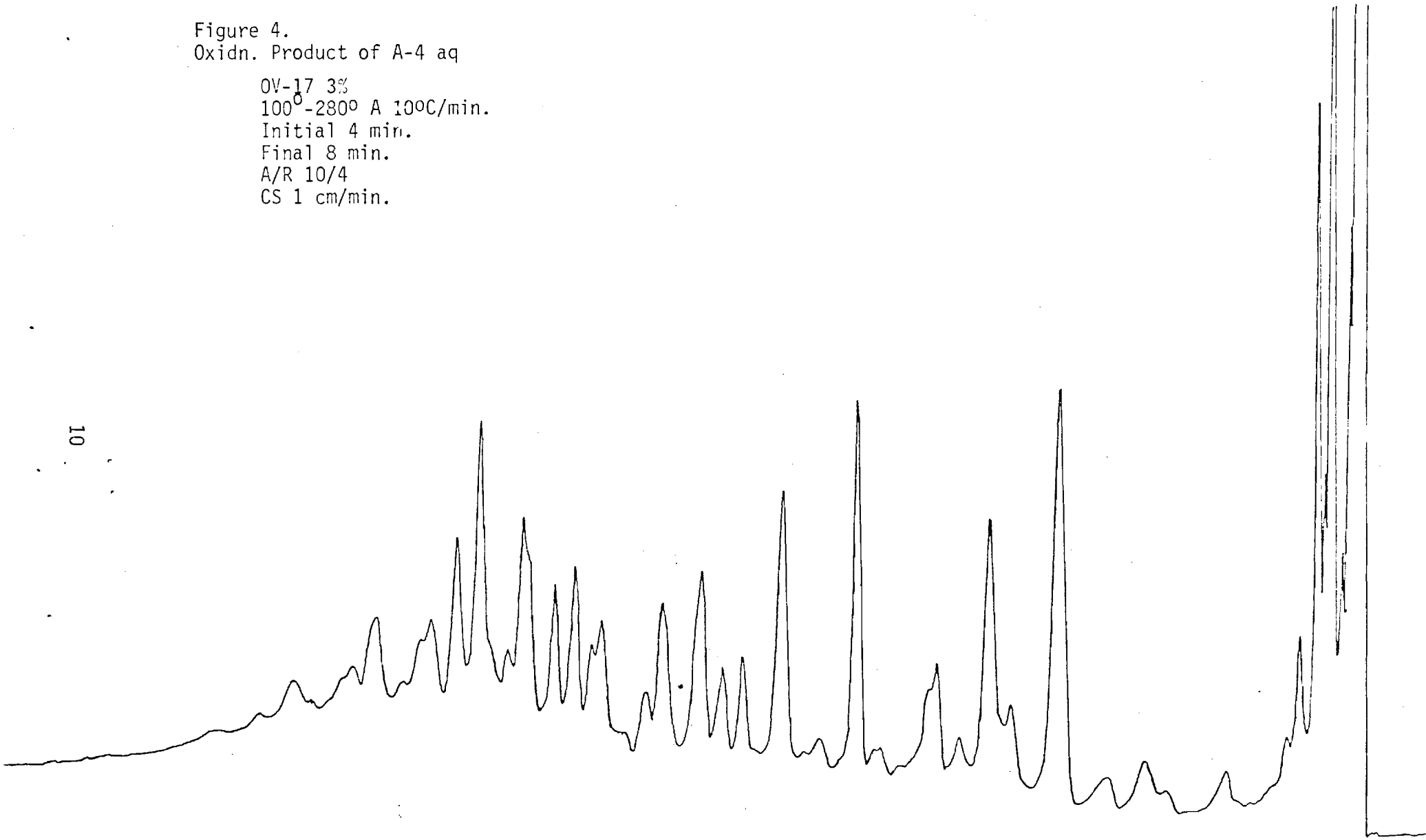
We are presently engaged in breaking up aquatic humics with neutral aqueous permanganate solution. We have also attempted to obtain proton and carbon-(13) magnetic resonance spectra to demonstrate how much aromaticity is exhibited by the aquatic humics.

Spectral Studies. A sample of river water humic matter was methylated with diazomethane. It became soluble in $CDCl_3$, and its proton magnetic resonance spectrum did not exhibit any aromatic protons. It showed peaks at δ0.66, 2.2 and a broad hump at δ3.87 in the ratio of 27:5:1. A very broad hump was spread out between δ0.77 and 1.7 (4H). No aromatic, aldehydic or acidic protons were discernible. Neither does IR spectroscopy 3000(sh), 2985(sh), 2950, 2845(sh), 1725, 1600, 1430 1250(br) cm^{-1} confirm aromatic character. A Fourier transform C-13 magnetic resonance spectrum showed only rather indistinct features even at a concentration of 300 mg/ml. However, this spectrum does show a small elevation at δ126 which might be due to aromatic carbon.

Oxidation. A 1g sample of river water humic matter was methylated with diazomethane to yield 1.18 g of methylated product. It was oxidized with aqueous $KMnO_4$ (4 w/v; 125 ml) by refluxing for 4 hours. All of the $KMnO_4$ was reacted, thus no additional reagent had to be introduced to destroy excess $KMnO_4$. The product (0.231 g) was methylated with CH_2N_2, and gas-chromatographic separation yielded 49 peaks (3% OV-17 on Gaschrom Q acid washed, silinized, 100-280^{o}C, 10^{o}C/min; FID—see Figure 4). Proton magnetic resonance spectroscopy shows peaks at δ4, 3.95, followed by a hump, 2.17 and 1.27. There was no indication of aromatic, acidic or aldehydic protons.

Figure 4.
Oxidn. Product of A-4 aq

OV-17 3%
100°-280° A 10°C/min.
Initial 4 min.
Final 8 min.
A/R 10/4
CS 1 cm/min.



The product was passed through a silica gel column and eluted with ethyl acetate to yield 144 mg of product. This is presumably free of high molecular weight unoxidized material. However, TLC on silica gel reveals material immobile in ethyl acetate.

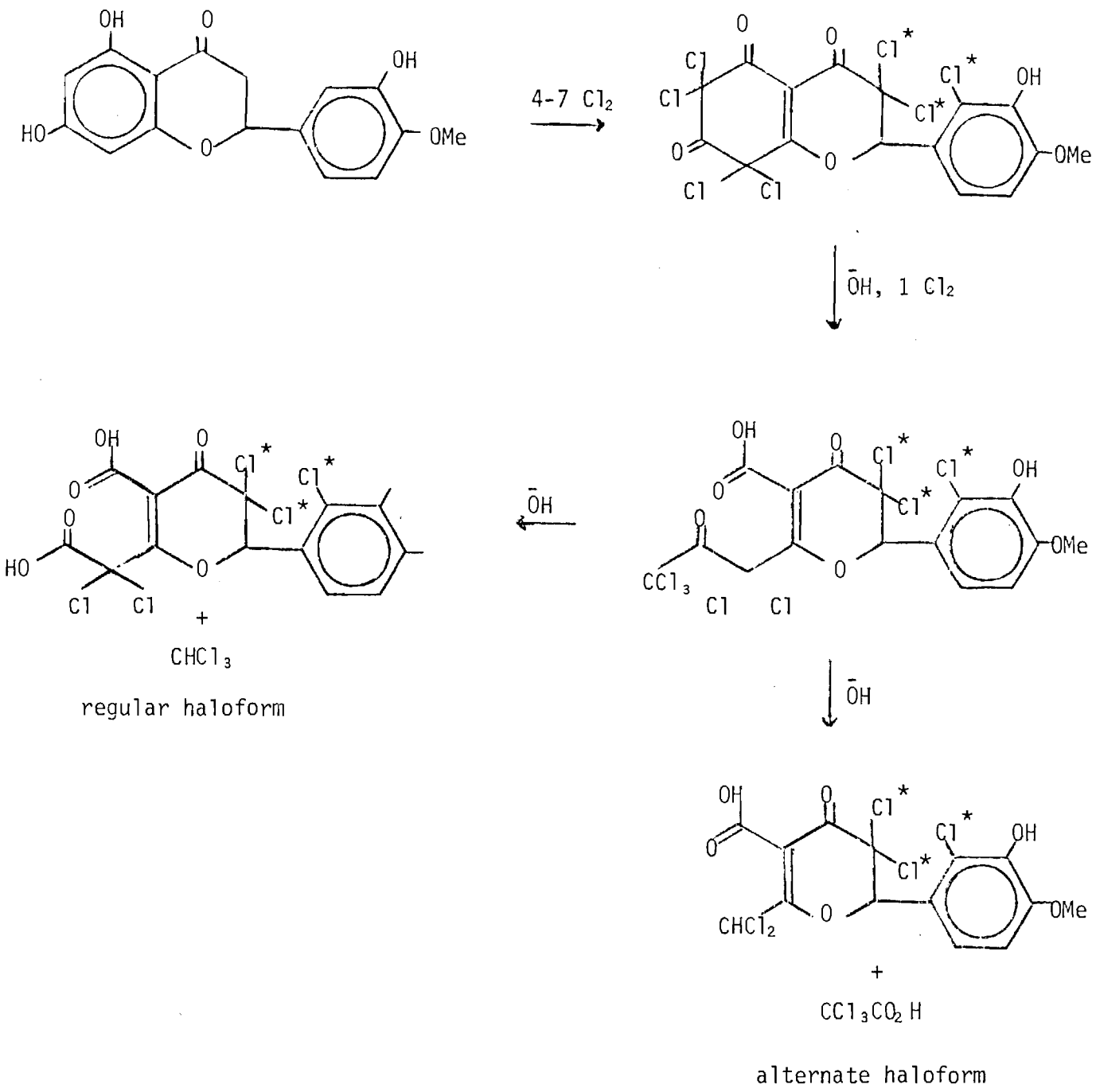
Although no definite conclusion can be drawn at this time, our observations seem to indicate that the aquatic humics on oxidation may not yield as great a proportion of benzenoid fragments as has been reported with similar substances by Schnitzer[3] and Ogner[4].

V. CHLORINATION OF MODEL COMPOUNDS

Hesperitin was dissolved in chlorine demand free water to the extent of 9.3 mg/l (3.1×10^{-5} molar). Upon treatment of this solution with 5.13 moles of Cl_2, transient development of color was noted. After 15 minutes, the original dose of chlorine had been completely taken up. An additional 2.34 moles of Cl_2 were added in stepwise fashion over the course of an hour after which time uptake had slowed to a negligible rate. Unless otherwise specified, this reaction and all others are carried out at ambient temperatures (about $20^{o}C$) and pH 7.5. The transient color development suggests the formation of an intermediate having extended conjugation or possibly a molecular complex. An attempt will be made to characterize this intermediate. Results for the trihalomethane analyses are not available as of this writing. Figure 5 shows a possible reaction mechanism based on the available evidence. In the near future, some effort will be devoted to the isolation and characterization of halogenated organic acids from this reaction mixture. Trichloracetic acid, if present, should be particularly easy to detect.

VI. PRELIMINARY STUDIES ON GENERATION OF DIAZOETHANE

A limited amount of work on generation of diazoethane from N-ethyl-N-nitrogen-N^1-nitroguanidine was completed. The diazoalkane generator described



Cl*

The exact point in the reaction scheme at which these Chlorines become attached is not clear. If all three are added 8 moles are required if two only 7 are required. Observed uptake is 7.5.

Figure 5. Tentative Mechanisms for the Reaction of Cl_2 With Hesperitin.

by H. M. Fales, T. M. Jaouni and J. F. Babashak[5] was purchased and used in two experiments. The micromole size unit will not accommodate sub-millimole scale reactions since the generation reaction is vigorous enough to drive the reaction mixture out the vent hole from which the diazoalkane is to escape into the outer receiver. The presence of strong sodium hydroxide in the ethereal diazoethane vitiated the benzoic acid titration method of diazoethane assay. In order to determine yields of diazoethane, the ethyl benzoate formed in the assay reaction was isolated and is in the process of being analyzed by gas chromatography.

For generation of larger amounts of diazoethane, the addition of solutions of N-ethyl-N-nitroso-N^1-nitroguanidine (this compound was a limited solubility in ethyl ether of about 0.6 g/100 ml) to a warm (bath temperature 50-55^{o}C), stirred, 5N aqueous sodium hydroxide solution gives a smooth generation of diazoethane and co-distillation with ether. The yield of diazoethane by this method appeared good as judged by the amount of benzoic acid required to discharge the yellow diazoethane solution. The measurement of actual yield also awaits gas chromatographic determination of ethyl benzoate.

VII. OZONE STUDIES

The generation of singlet oxygen from hypochlorite and hydrogen peroxide according to the method of Foote and Wexler[6] was investigated further. Chemical generation has been successful based on the observation of chemiluminescence in the reaction mixture. Our ability to observe a corresponding luminescence from the photochemical ozone generator has been hindered by stray light emitted by the UV source. We are currently devising a means of circumventing this difficulty. The reactivity and transient nature of the activated oxygen species will make this a difficult task.

Photochemical "ozone" is a strong enough oxidant to produce chlorine from chloride at pH 6.4 according to the neutral orthotolidine method. Chlorine dioxide was not produced in amounts sufficient for detection via UV-VIS spectroscopy. Chlorine dioxide could be generated, however, from chlorite at pH 6.8 and 11.0.

Photochemical ozone was reacted with resorcinol at concentrations of 19.2 mg/l and 100 mg/l. The decomposition was followed by the decrease in ultraviolet absorption at 205 and 276 nm. Figure 6 shows typical results. The shape of the curves suggest a free radical reaction featuring an initiation period or an antocatalyzed reaction. More definitive work is in progress.

A more qualitative experiment was run using resorcinol (approximately 50 mg/l) and photochemical ozone. Sodium nitrate (approximately 100 mg/l) was added to one-half of the sample so that a comparison could be made of the relative rates of decomposition of resorcinol with and without added nitrate. This reaction was undertaken at the suggestion of one of our colleagues in the Chemistry Department[7] on the basis of an analogy with published work on the radiolysis of aqueous solutions of N_2O. The results are shown in Figure 7. A similar experiment with nitrite was inconclusive.

More quantitative experiments are planned for the near future. A comparison will be made between the three different types of photochemical ozone generators which are now on hand, the Union Carbide type generator and the Welsbach generator. Another series of experiments will compare the relative ability of ozone, chlorine and chlorine dioxide to decompose a series of model compounds.

Figure 6. Photochemical Ozone-Induced Decomposition of Resorcinol-I

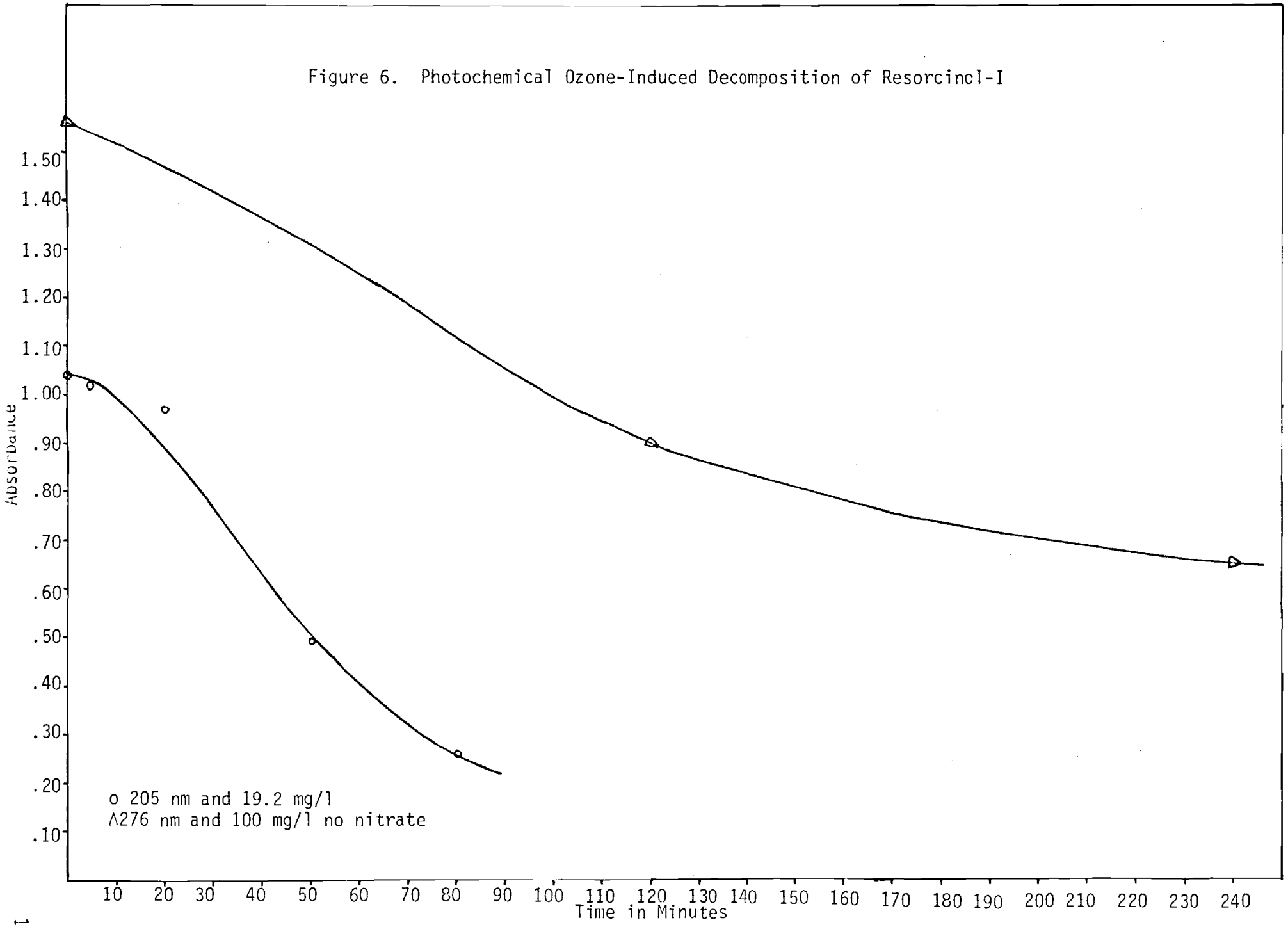
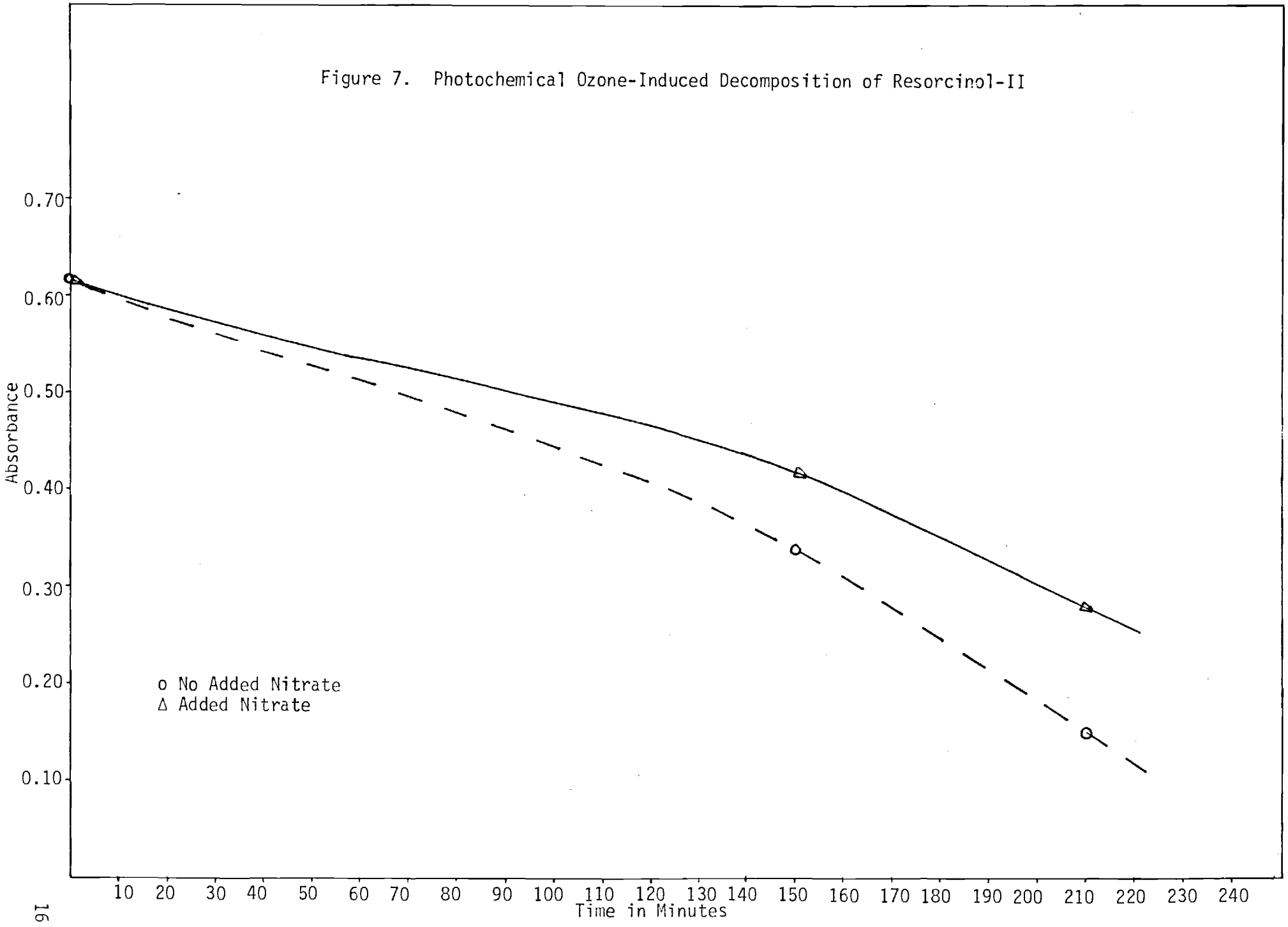


Figure 7. Photochemical Ozone-Induced Decomposition of Resorcinol-II



VIII. CONTINUED WORK WITH CARBOHYDRATE GC

The June 5th monthly report contained some preliminary results of the GC separation of sugars as the trimethylsilyl (TMS) ether derivatives. Subsequent work has yielded the separation of TMS-mono and TMS-disaccharides shown in Figure 8. Table II gives retention times for the TMS derivatives. The derivatives were prepared by dissolving a few mg of the pure sugars in TRISIL-Z (Pierce Chemical Co.) while heating at 70°C. The Pierce Co. states that reactions in TRISIL-Z are usually complete upon dissolution of the compound. Glucose and galactose, however, do not react completely upon dissolution at 50°C. Chromatograms of TMS-glucose and TMS-galactose produced by reaction at 50°C contained multiply peaks, while derivatives formed at 70°C resulted in single major peaks. Chromatograms of galactose derivatives formed at the two temperatures are shown in Figure 9.

IX. PRELIMINARY STUDIES WITH CHLORINE DIOXIDE

A mixture of resorcinol (100 mg/l) and chlorine dioxide (less than excess) was examined by the methods of UV-VIS spectroscopy in order to pinpoint the nature of the strong red-violet coloration which develops immediately upon mixing the reactants. Under these conditions, the absorbance due to ClO_2 at 362 nm was absent and the absorption of resorcinol at 276 nm was off-scale. A new absorbance was seen at about 505 nm. This is believed to be the ClO_2—resorcinol molecular complex of some other kind of reaction intermediate. This phenomenon of immediate color formation with phenolic compounds seems to be a general one and would seem to be worthy of further investigation. Both chlorine and chlorine dioxide have been observed to show this color development. (Hesperitin turns deep yellow with Cl_2.)

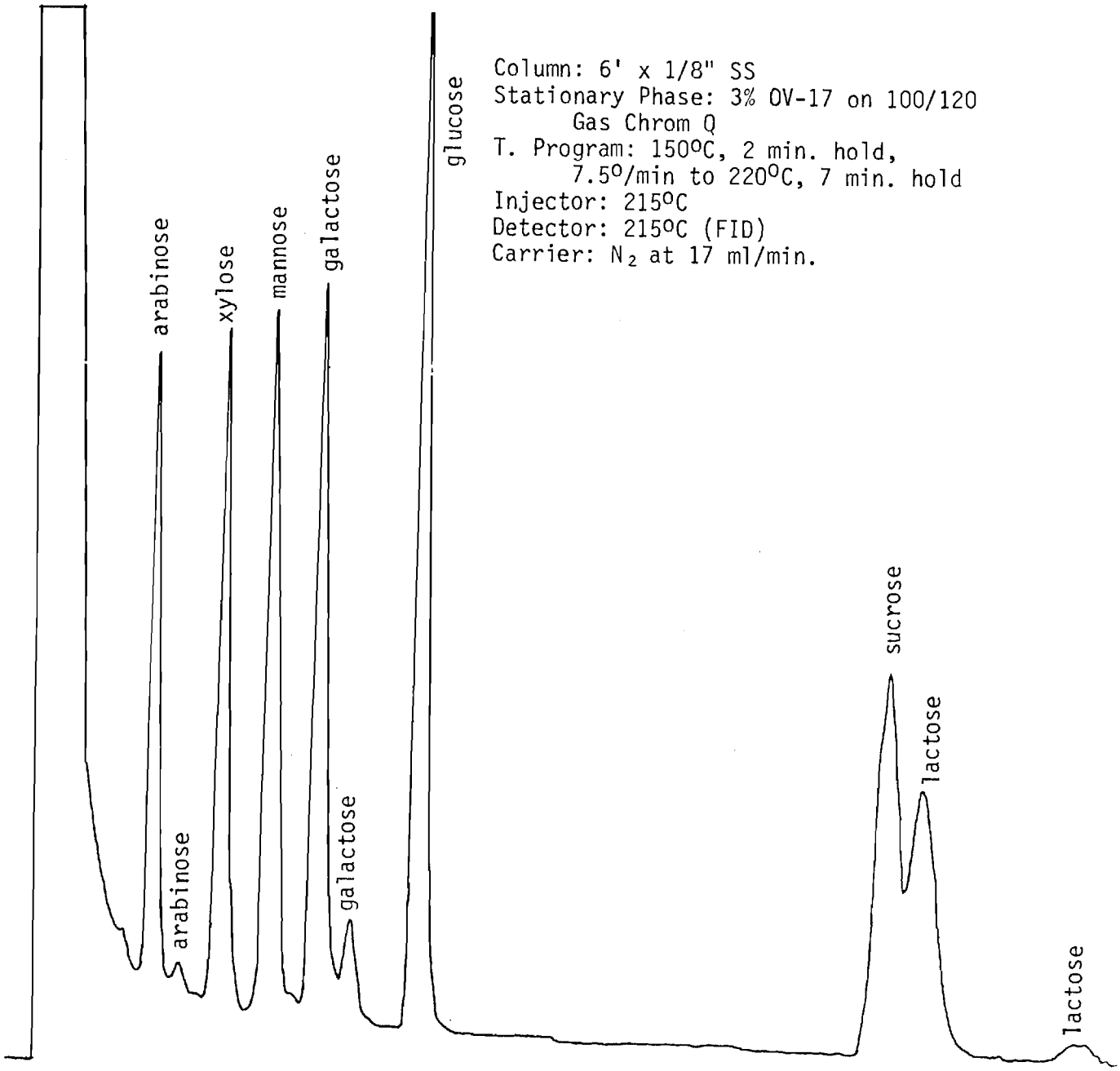


Figure 8. Gas Chromatographic Separation of Sugar Trimethylsilyl Ethers.

Table II

Retention Times of Trimethylsilyl Ether Derivatives of Some Mono and Disaccharides

Sugar	Retention Time	
	Minutes	Relative to Glucose
Arabinose	2.0, 2.5	0.30, 0.38
Xylose	3.2	0.48
Mannose	4.0	0.61
Galactose	4.9, 5.3	0.74, 0.84
Glucose	6.6	1.00
Sucrose	14.6	2.21
Lactose	15.2, 16.2	2.30, 2.45

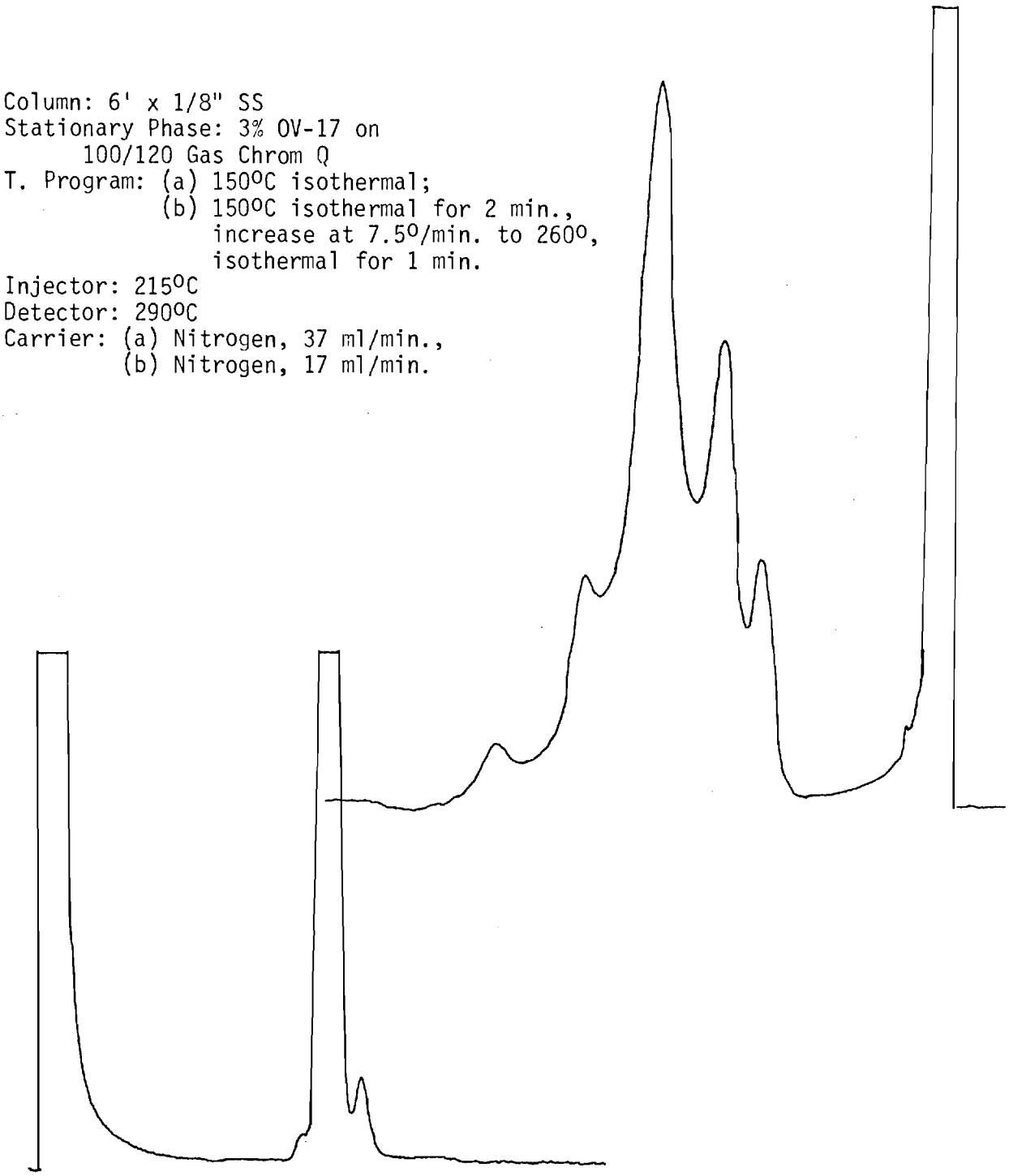


Figure 9. (a) Chromatogram of TMS-Galactose Derivative Formed by Reaction at 50°C.
(b) Chromatogram of TMS-Galactose Derivative Formed by Reaction at 70°C.
The chromatograms were obtained using different recorders, and under slightly different chromatographic conditions.

References

1. Husek, P. and K. Malek, J. Chromatog. 113, 139 (1975)
2. Roach, D. and C. W. Gehrke, J. Chromatog. 44, 269 (1969).
3. Schnitzer, M. and M.I. Ortiz de Serra, Soil Biol. Biochem. 5, 287 (1972).
4. Ogner, G. and E. T. Gjessing, Geoderma 14, 139 (1975).
5. Fales, H. M., T. M. Jaouni and J. F. Babashak Anal. Chem. 45, 2302 (1973).
6. Foote and Wexler, J. Am. Chem. Soc. 86, 3879 (1964).
7. W. H. Eberhardt, personal communication.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

September 2, 1977

by

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68-01-4480

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DISCLAIMER

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I. PERSONNEL

No personnel changes have taken place during this reporting period.

II. EQUIPMENT

A new Torbal SEA-1 analytical balance has just been uncrated and is now being set up directly in the laboratory. This will improve laboratory efficiency by eliminating the need for time-consuming trips to adjacent facilities. The GC/MS system is somewhere between California and Georgia and should (the manufacturer swears) be in place by the time this report reaches the sponsor. All of these general purpose items have been purchased out of Station funds and are mentioned only in the context that they will have a favorable impact on the sponsor's research program.

The order for the attenuator stand and scanning accessory for the LC has been held up for over a month pending the approval of Mr. Bailets (EPA) regarding the shift of "materials and supplies" funds to "equipment." Since the original proposal makes it clear how much is to be spent for equipment, we do not understand what the problem is. (We do acknowledge the fact that getting these items listed as supplies in the first place was our mistake, but LC accessories were specifically mentioned, and therefore the request is not "new" to the sponsor.) This equipment will permit us to obtain a complete UV-VIS spectrum of individual LC peaks as they elute from the instrument. Information of this type should be very helpful in promoting an understanding of structural changes which are going on in our factorial experiments.

A corona type ozone generator has been obtained and partially rebuilt to eliminate possible contamination resulting from heat induced volatilization

of plasticizers from the original plastic casing.

III. GAS CHROMATOGRAPHIC STUDIES

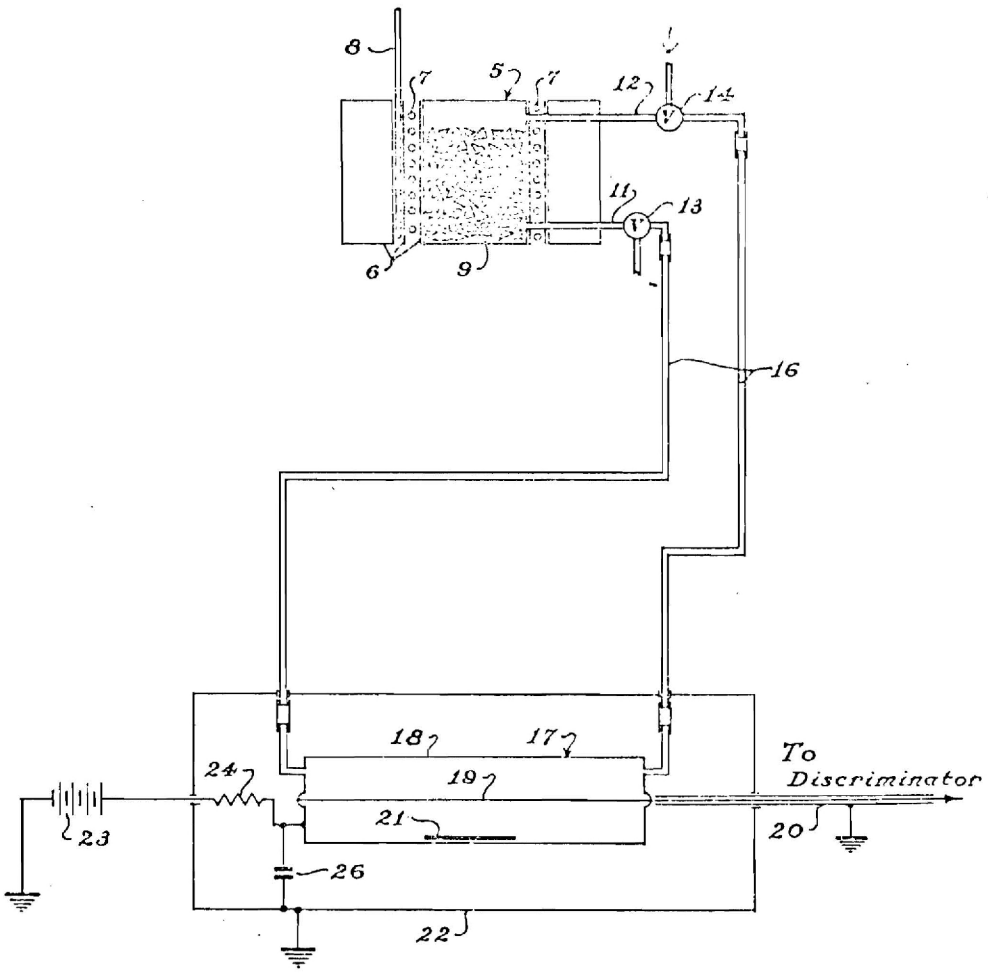
Since some project funds have been devoted to the task of fitting Mr. Lupton's unique electron capture (porous anode) detector to our gas chromatographic instrumentation, it is deemed appropriate to begin this section with a brief description of the design of this detector and some of its capabilities. We have already pointed out one of its non-standard features—namely the ability to recover rapidly after the injection of a chlorinated solvent so that full advantage can be taken of the superior extraction properties of such solvents, for halogenated materials (page 3, last progress report).

The use of flow-through ionization chambers was reported by E. D. Klema in his patent of April 1951 in which he used such a device as part of a system designed to prepare pure hydrogen which would be free from water, oxygen and halogens. Figure 1 is a pictoral schematic of Klema's device. It is important to note that the inlet and outlet of his system is interchangeable. In this case the inlet gas sample is allowed to mix in the chamber in a random manner. Modern "electron capture" devices do, however, direct the gas from the cathode to the anode. An example of this is found in Analytical Chemistry, Volume 43, No. 14, December 1971, in an article by D. C. Fenimore and P.R. Loy. Figure 2 clearly shows the cathode-to-anode gas flow. Unfortunately this chamber design allows the gas sample to recirculate within the chamber for some time rather than taking a more direct, single-pass route such as is possible in the Lupton device. Figure 3 shows the passage of the gas from the anode to the cathode which is one of the unique features of the Lupton detector.

April 3, 1951 E. D. KLEMA 2,547,874

HYDROGEN PURIFICATION METHOD

Filed May 24, 1948



WITNESSES *INVENTOR.*

Figure 1

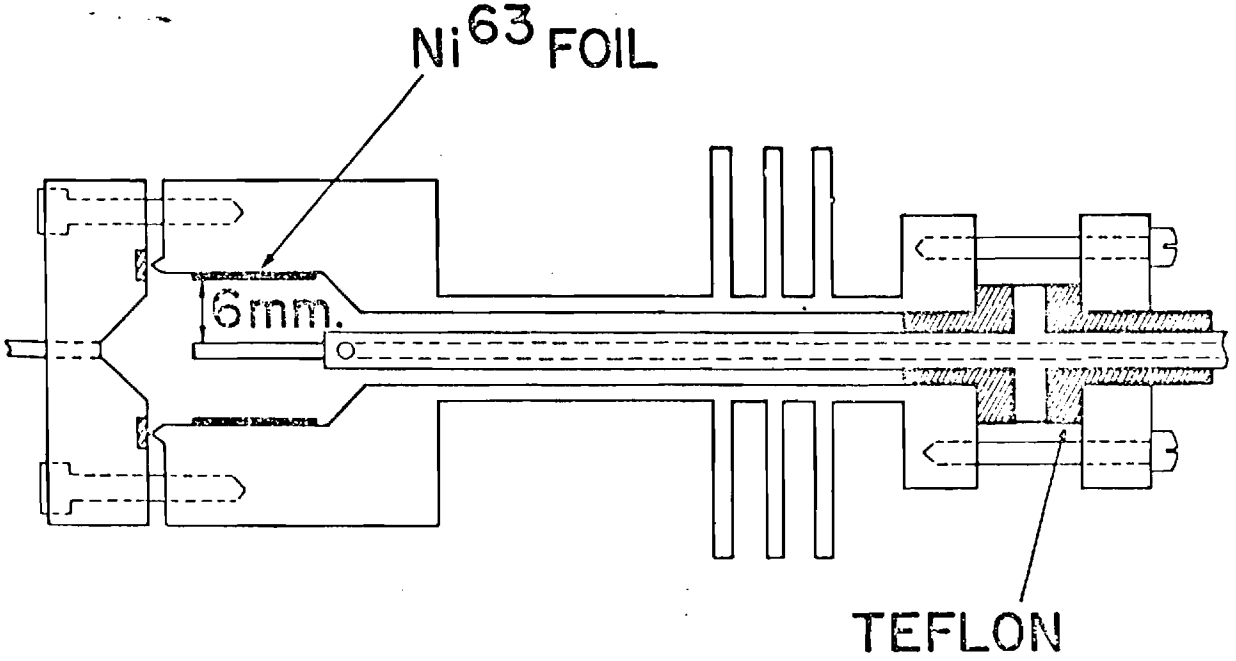
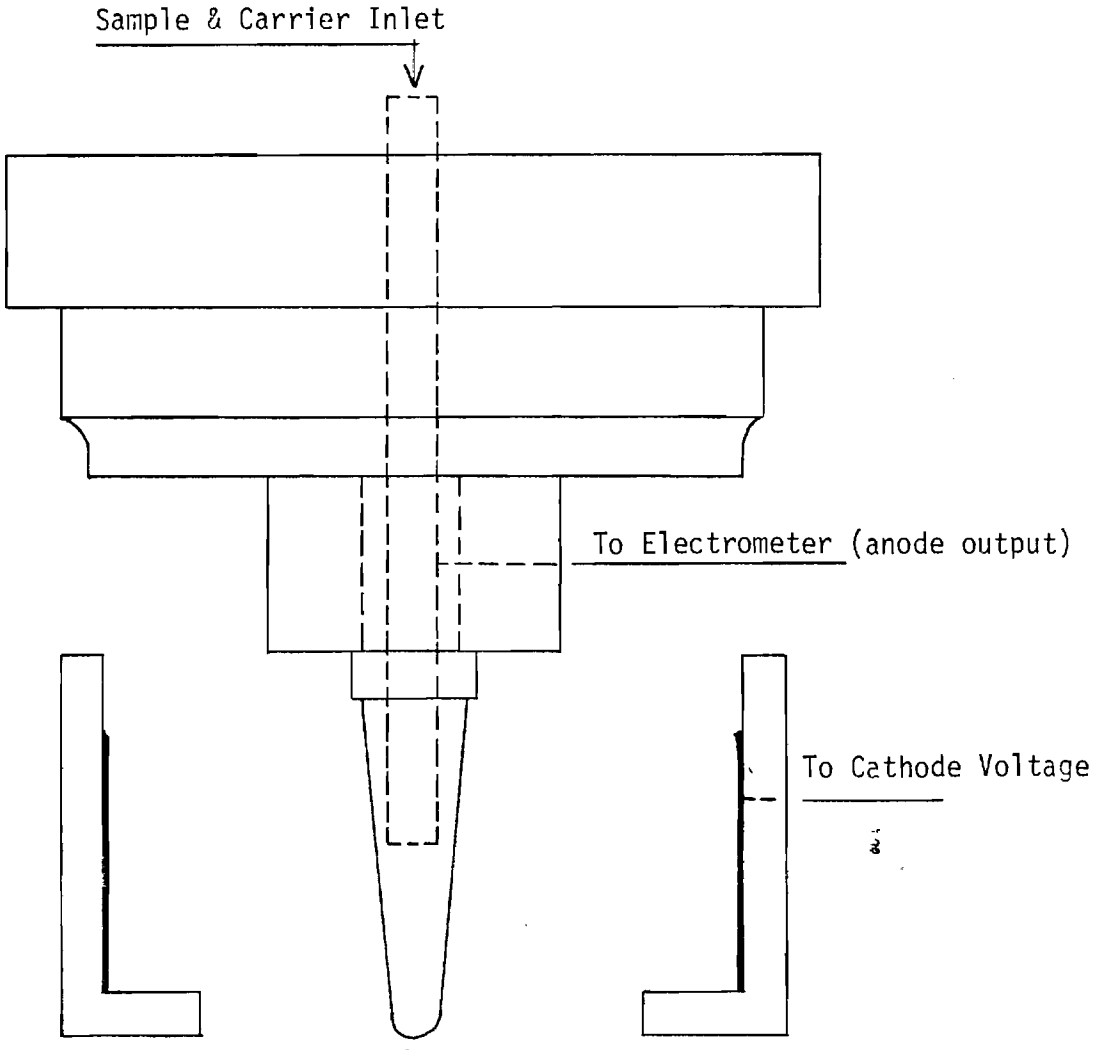


Figure 2. High temperature electron capture detector.

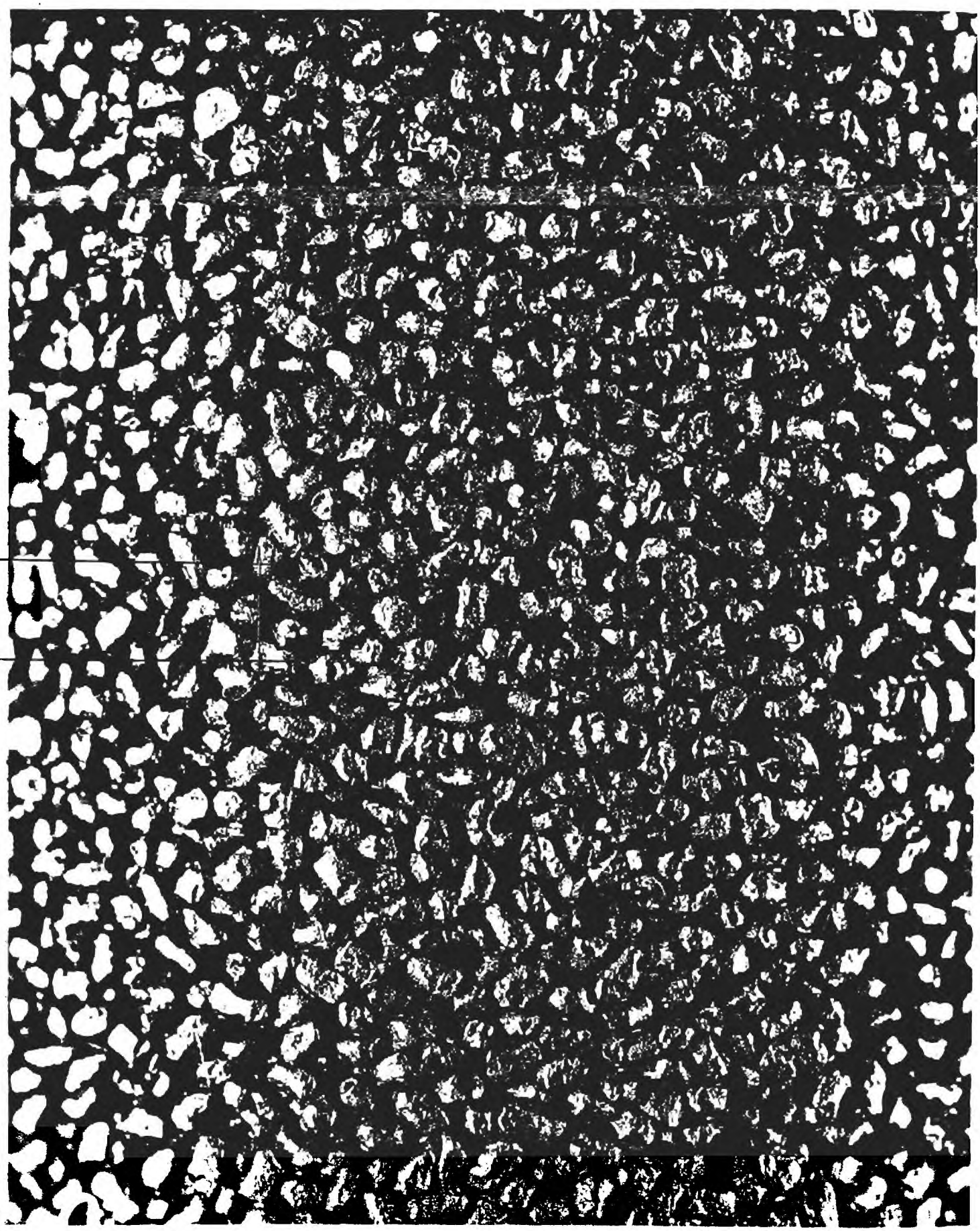


Lupton Detector
Figure 3

Since the gas has a greatly reduced opportunity for recirculation, the Lupton detector is not "swamped" by halogenated solvents.

Beta particles from the Ni^{63} foil irradiate both the surface and sub-surface of the anode as the carrier gas and sample diffuse through the porous surface. Figure 4 is a photomicrograph of a typical anode (unplated). Ionization by beta particles of carrier gas and organic sample takes place between the small anode chips as seen in Figure 4. Just how deeply this ionization takes place is not known at this time. After ionization the gas mixtures collide with the anode chip and electrical current changes are noted. This entire process takes place just beneath the anode surface. After the gases leave the surface they are free to migrate out of the chamber. Continuous gas flow from within the anode prevents recirculation. The anode end of the chamber is open to the atmosphere through a 0.35 inch opening. The area between the anode and cathode is therefore at atmospheric pressure. Figure 5 is the first page of a patent by J. E. Lovelock in which sample and carrier gas also enter the ionization chamber against the beta flow. Furthermore it is interesting to note the changes in response direction and changes in sensitivity as the cathode voltage is changed. In our own work, reproducible runs have been made with the cathode voltage as low as 0.200 volts DC.

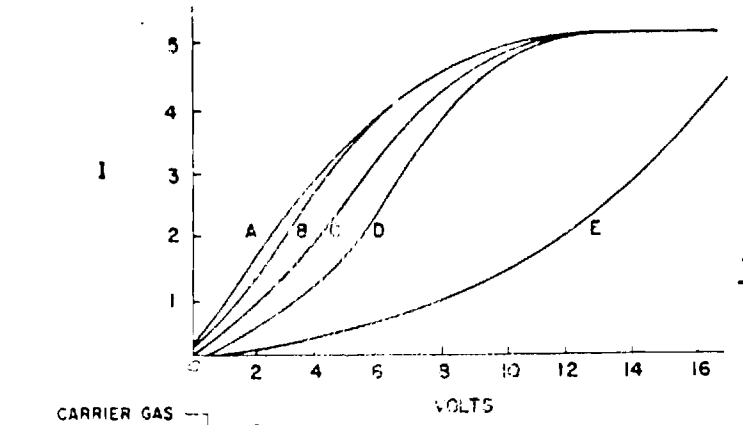
This brief description is intended as an overview only and is meant to explain the basic reasons for the ability of the Lupton detector to successfully perform even when chloroform is used as a solvent. We plan to take advantage of the high degree of sensitivity of this detector for the detection of trihalomethanes. It offers a reliable, inexpensive system which is easier to operate than conventional electron-capture detectors.

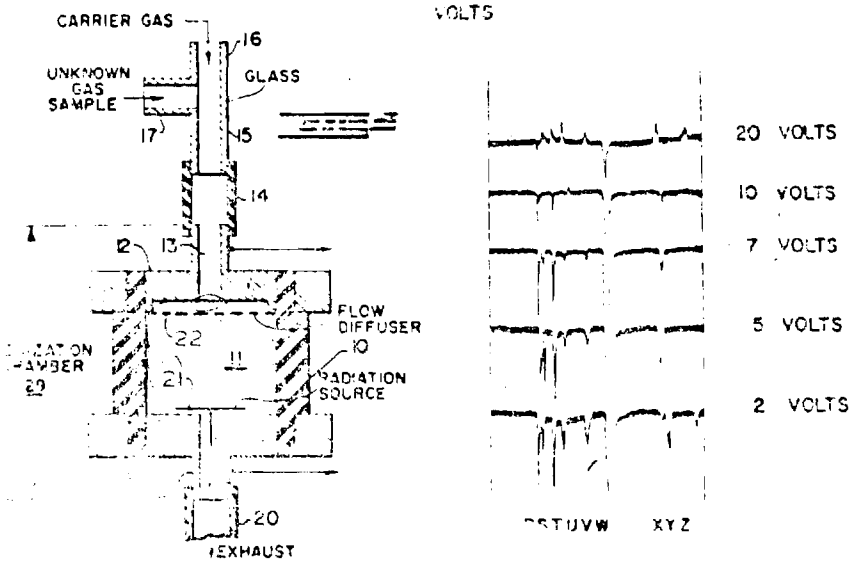


April 19, 1966 J. E. LOVELOCK 3,247,375

GAS ANALYSIS METHOD AND DEVICE FOR THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF CLASSES OF ORGANIC VAPORS

Filed Dec. 23, 1960 2 Sheets-Sheet 1





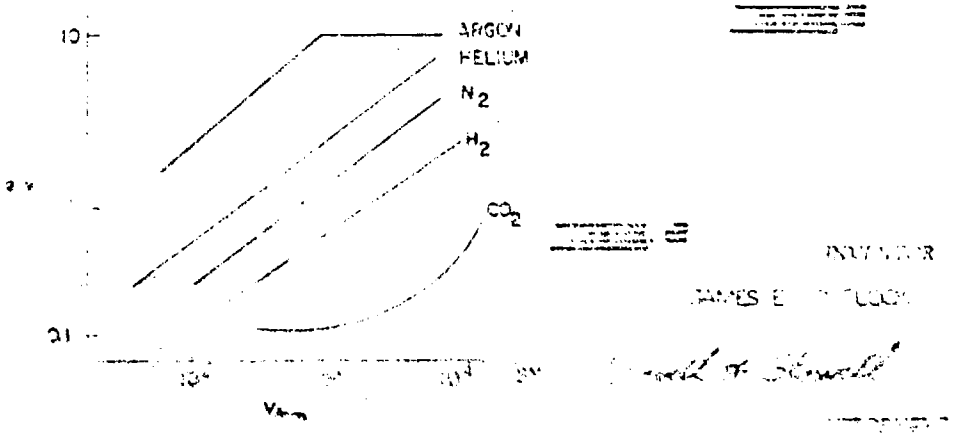
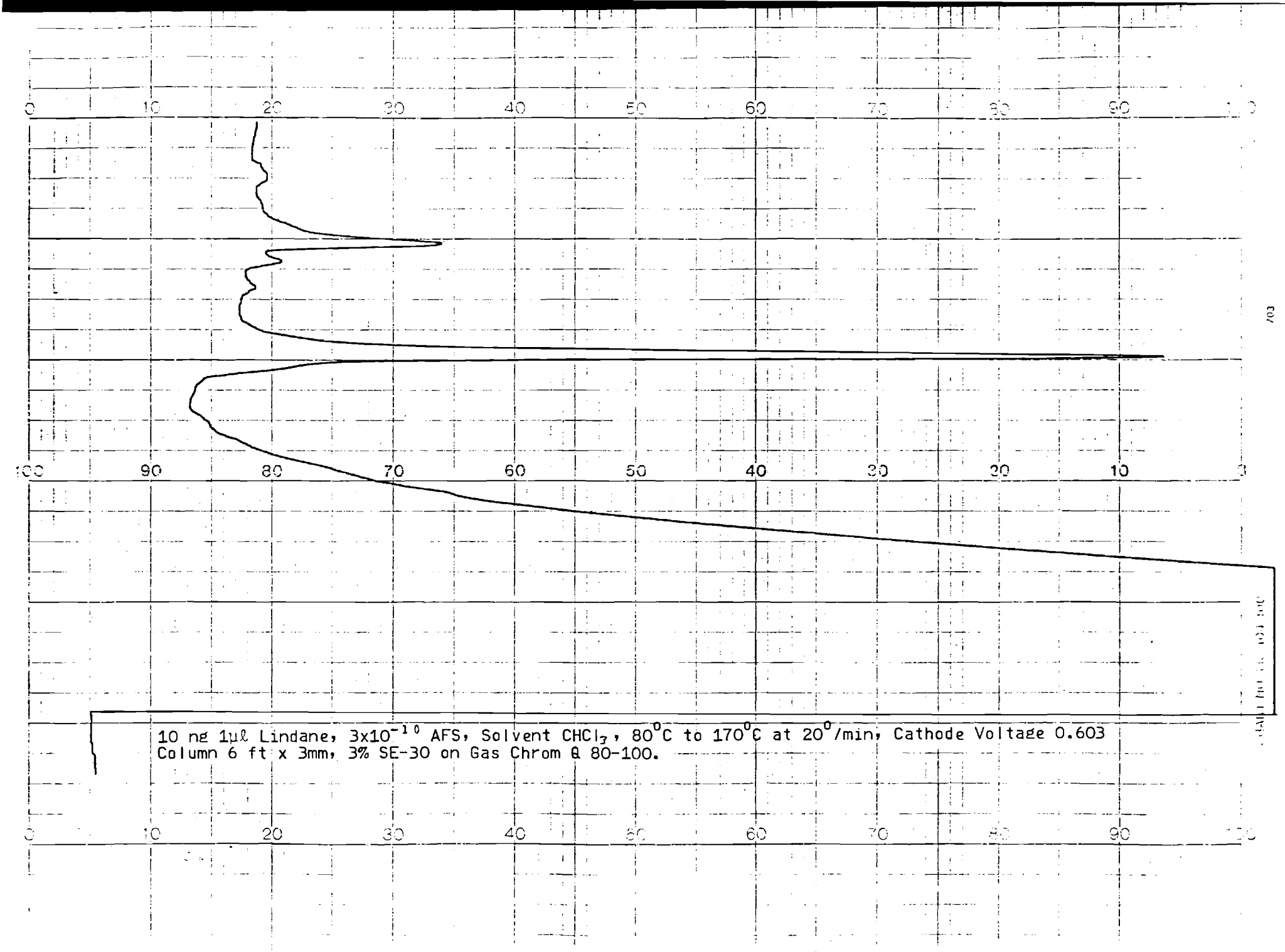


Figure 5

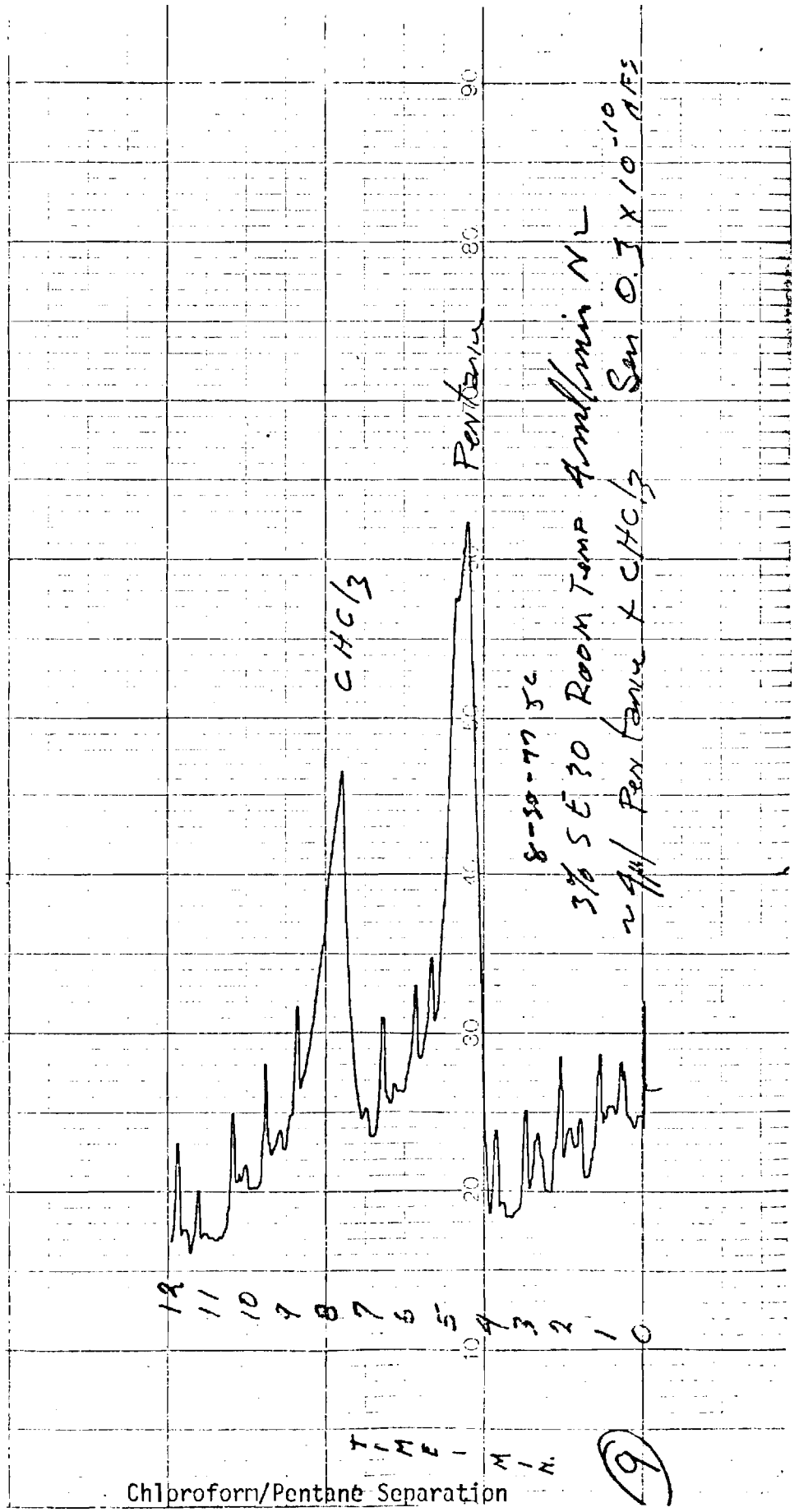
Figure 6 shows 10 nanograms of lindane being separated from 1 μℓ of chloroform. Temperature programming is a useful tool for separations of this type. Unfortunately, instrumental drift during rapid temperature programming is a common failing of many EC detection systems. The conditions chosen ($20^{o}C$/min) were deliberately selected to present an extreme case. It would appear that baseline drift is negligible. However, other data used to prepare baseline vs. temperature curves show a somewhat greater drift than is indicated by this curve. A possible explanation for the extra degree of drift deppression seen in Figure 6 may be due to a combination of negative drift from the chloroform and positive drift from the changing column temperature.

Pentane and $CHCl_3$ were separated (Figure 7) with 3% SE-30 on Chromsorb W (80/100 mesh). The 6' x 3 mm ID glass column was operated at room temperature with a nitrogen flow of 4 mℓ per minute. The peak shape is poor because the column is not optimized for maximum theoretical plate efficiency of this column. A capillary column operated under these conditions would be expected to generate peaks of much better shape. This graph does show the practicability of operating this EC detector at low flow rate conditions favorable to capillary columns.

Detector performance was characterized by injecting a series of 1-3 μℓ samples of lindane—the concentrations of which were precisely known. Plots of peak height vs. nanograms of lindane (see Figure 8) and peak area vs. nanograms of lindane (see Figure 9), were prepared. These data indicate that the Lupton detector is comparable to other "electron-capture" devices in sensitivity and linearity of response. Thus, without any apparent sacrifice in detector response, these advantages are realized: (1) the capability of accommodating the direct injection of halogenated solvents, (2) the ability of perform rapid temperature programming with minimal drift and (3) the capacity to work effectively at very low flow rates.



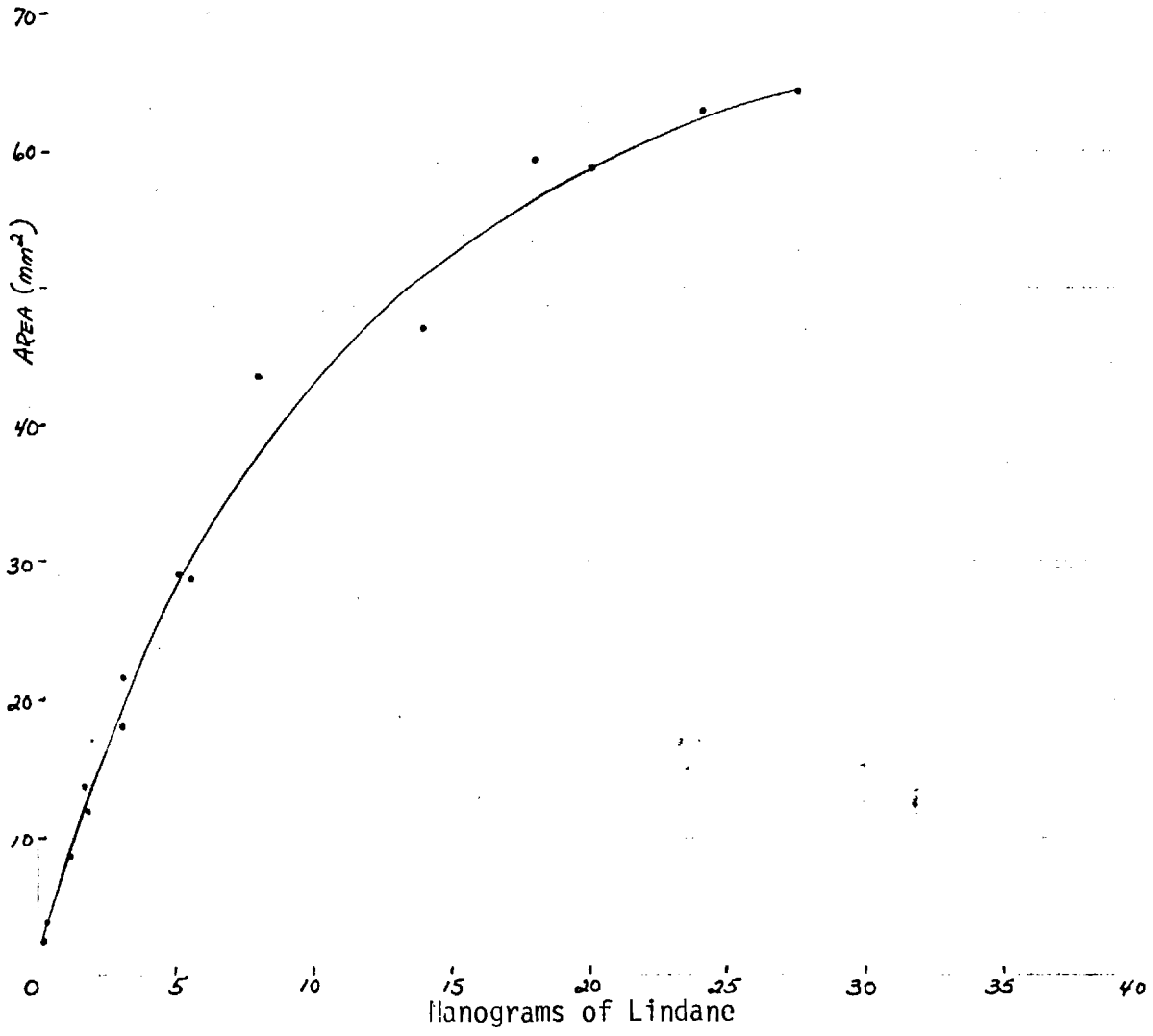
10 ng 1µℓ Lindane, $3x10^{-10}$ AFS, Solvent $CHCl_3$, 80°C to 170°C at 20°/min, Cathode Voltage 0.603
Column 6 ft x 3mm, 3% SE-30 on Gas Chrom Q 80-100.



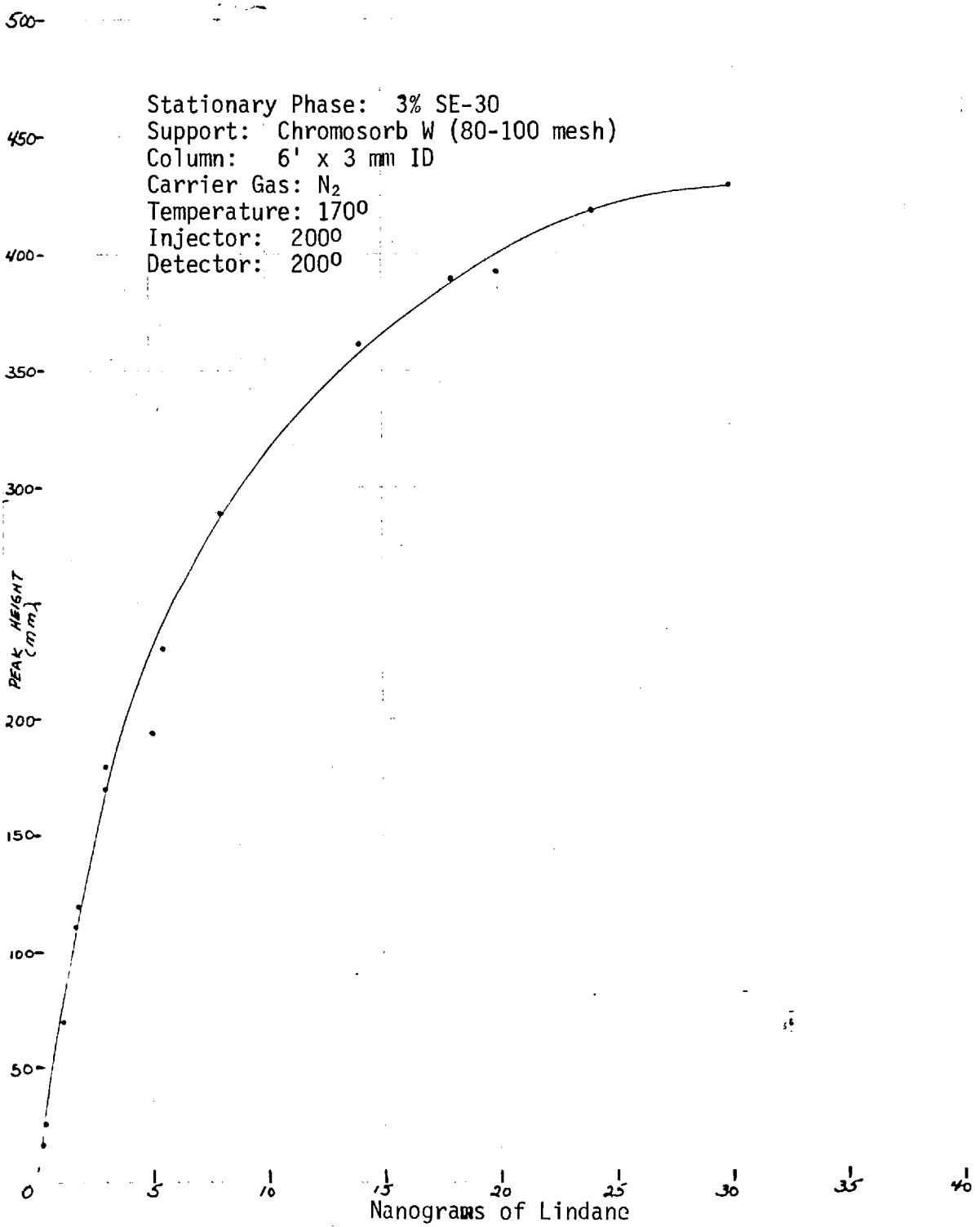
Chloroform/Pentane Separation

Figure 7

Stationary Phase: 3% SE-30
Support: Chromosorb W (80-100 mesh)
Column: 6' x 3 mm ID
Carrier Gas: N_2
Temperature: 170°
Injector: 200°
Detector: 200°



Peak Area as a Measure of Detector Response
Figure 8



Peak Height as a Measure of Detector Response
Figure 9

Since uracil is a representative constituent of the biologically significant nucleic acids which has further been demonstrated to react with chlorine under conditions encountered in typical water and wastewater treatment processes, we were delighted to learn that Dr. J. P. Gould of our Department of Civil Engineering was studying this very question. He has graciously supplied us with samples of uracil and 5-chlorouracil so that we might characterize these substances by LC, GC and GC/MS. In this way, we will learn more about where to find these substances in our isolation schemes and what their behavior will be when they are examined using our instrumental methods and he will have needed confirmatory evidence regarding the identity of various constituents in his reaction mixtures.

As of this writing, our reports have been confined to our experiment using the techniques of gas chromatography. An attempt was made to prepare the trifluoroacetyl derivatives using trifluoroacetyl anhydride (TFAA) (2:1 CH_2Cl_2: TFAA at 150^0 for 15 minutes). This treatment was followed by gas chromatographic analysis on a 2% OV 17/1% OV 210 column previously described in the monthly report for July (pages 4-5) . The samples derived from uracil and 5-chlorouracil both showed the same retention time and it is therefore uncertain if the TFAA reacted with either compound at all, displaced the 5-chloro substituent or if the conditions of separation were just not capable of separating the two compounds.

Additional work will be done with the compounds using direct injection without derivatization on a OV-17 column which will permit temperature programming to a higher temperature than that possible with columns containing OV-21. Mass spectrometry can be expected to clear up the questions of whether or not these materials will react with TFAA under the mild conditions employed.

Attempts to prepare trimethylsilyl ethers of hersperitin and coniferyl alcohol have not been successful thus far. A more powerful silanizing reagent "Trisil-z" (trimethylsilyl-imidazole in pyridine) has been ordered due to reports in the literature that this a most effective reagent for the silylation of flavonoids J. Chromatog. 121, 49 (1976) .

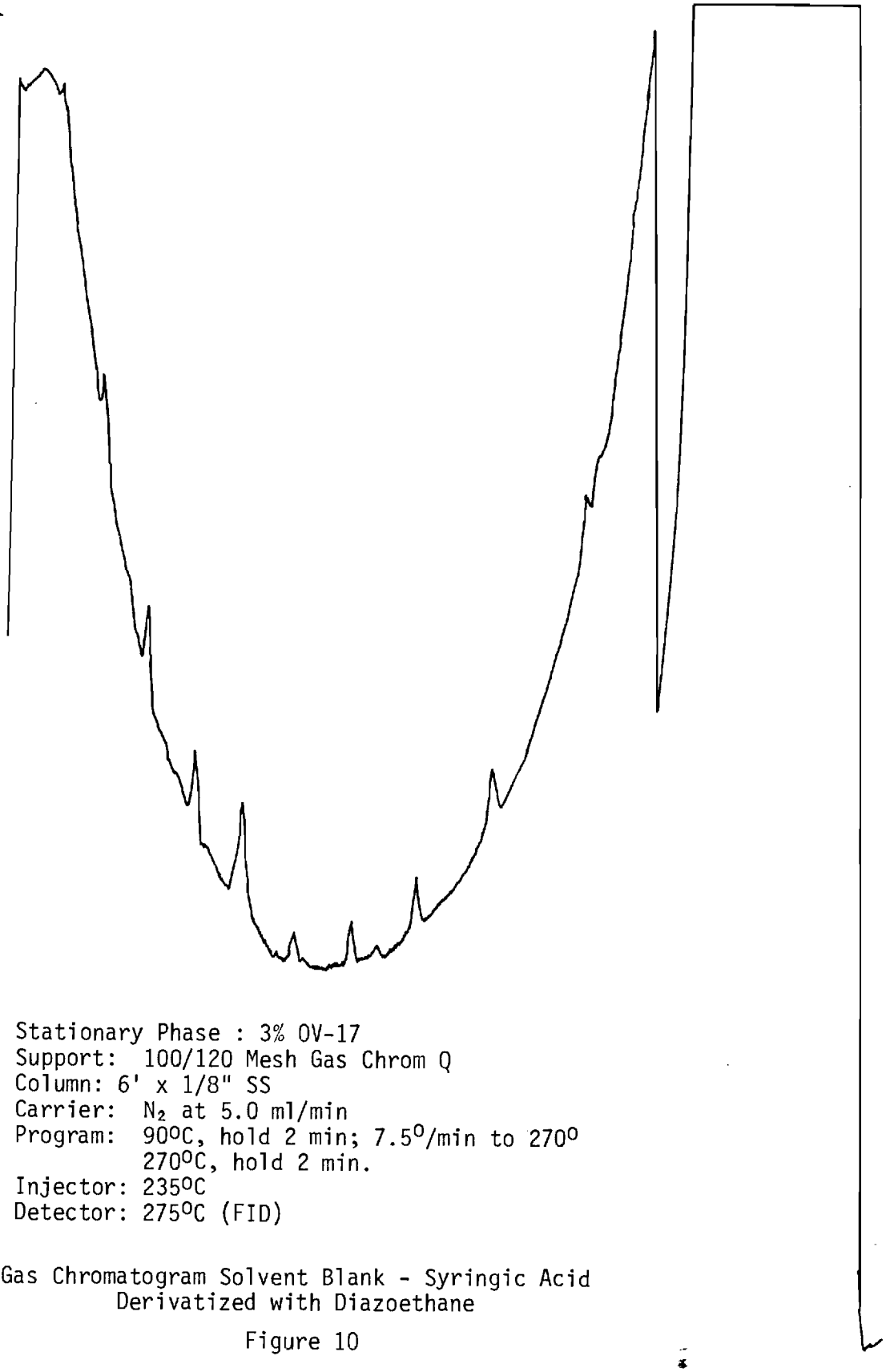
Some preliminary results were obtained regarding the gas chromatography of reaction products from the factorial series of coniferyl alcohol chlorinations. The pentane extract concentrates containing neutral compounds was examined for compounds having a lesser degree of volatility than the trihalomethanes. A 3% OV-1 column (6 foot of 1/8 inch), stainless steel was used with the following temperature program:

Hold at 100°C for 1 minute.

Hold at 290°C for 3 minutes.

Results thus far are inconclusive.

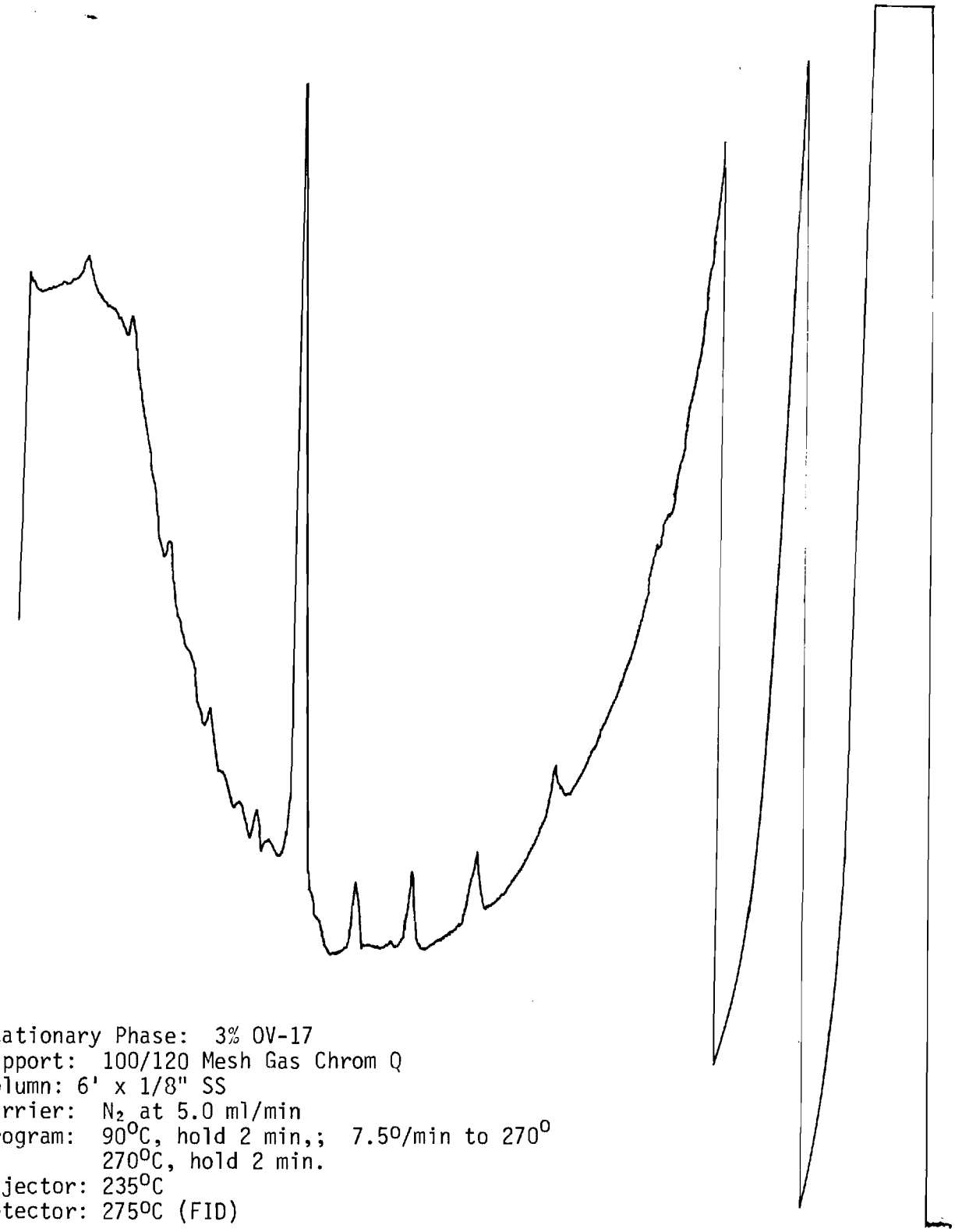
Developmental work has also been carried out regarding the gas chromatographic behavior of coniferyl alcohol and syringic acid when derivatized with diazoethane. On the basis of this work, it is evident that impurities will be a problem at the high sensitivities required for the analysis of trace quantities of these substances. This problem is illustrated by the presence of what appears to be a homologous series in the solvent blank as shown in Figure 10. Nevertheless, a good separation of the derivatized syringic acid from the background interferences could be achieved as is illustrated by Figure 11. The attempted derivitization of coniferyl alcohol did not yield any products which were capable of being separated or detected under these conditions. It should also be pointed out that is is important to regularly



Stationary Phase : 3% OV-17
Support: 100/120 Mesh Gas Chrom Q
Column: 6' x 1/8" SS
Carrier: N_2 at 5.0 ml/min
Program: 90°C, hold 2 min; 7.5°/min to 270°
270°C, hold 2 min.
Injector: 235°C
Detector: 275°C (FID)

Gas Chromatogram Solvent Blank - Syringic Acid
Derivatized with Diazoethane

Figure 10



Stationary Phase: 3% OV-17
Support: 100/120 Mesh Gas Chrom Q
Column: 6' x 1/8" SS
Carrier: N_2 at 5.0 ml/min
Program: $90^{o}C$, hold 2 min,; 7.5^{o}/min to 270^{o}
$270^{o}C$, hold 2 min.
Injector: $235^{o}C$
Detector: $275^{o}C$ (FID)

Gas Chromatogram Syringic Acid Derivatized with Diazoethane

Figure 11

dose the column with a silanizing conditioner such as silyl-8 in order to achieve reproducible results under conditions of maximum sensitivity.

IV. SAMPLES AND PRELIMINARY SEPARATIONS - HUMIC STUDIES

A sample of river water humic matter from the Satilla River was fractionated by gel permeating chromatography. The extractions were begun with Sephadex G-50 and followed consecutively by G-25, G-15 and G-10. Five fractions were obtained: (I) G-50 excluded; (II) G-25 excluded; (III) G-15 excluded; (IV) G-10 excluded; and (V) retarded. Fraction II (G-25 excluded) contained almost half of the starting material. Its number average molecular weight, determined by vapor pressure osmometry , was 2224. Oxygen-containing functional group analysis gave 10.6 meq/g total acidic protons, divided into 6.1 meq/g carboxyl and 4.5 meq/g phenolic hydroxyl groups. Elemental analysis gave 51.4% carbon, 3.6% hydrogen, 0.7% nitrogen and (calculated by difference) 44.2% oxygen.

One g of fraction II was methylated with diazomethane and oxidized with 4% aqueous permanganate solution (4 hrs, reflux). The pH of the solution rose to 10. After filtration to remove MnO_2 the solution was acidified to pH 2 and extracted (liquid/liquid) for 72 hrs with ethyl acetate. After evaporation of the solvent the product was remethylated with diazomethane to yield 0.22 g of product.

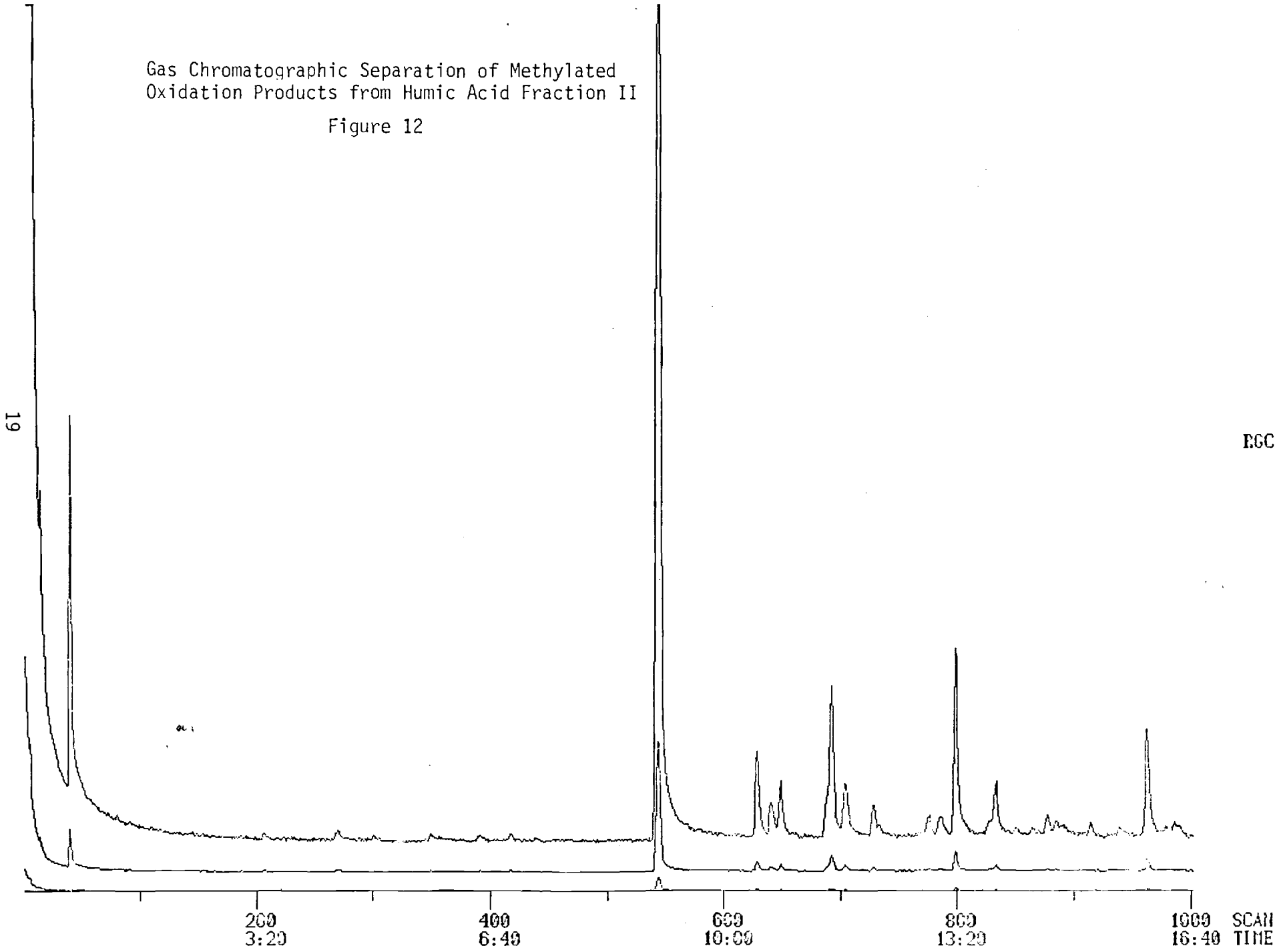
A gas chromatogram of this oxidation product on a 6' x 1/8" column packed with 3% OV 17 on Chromosorb AW shows 64 peaks. (See last monthly report page 10.) Using a glass capillary column (150 x 0.02", coated with OV 101), the same mixture resolved into 119 peaks. This chromatogram is presented in Figure 12. Mass spectra taken of the effluent materials from this column have been recorded and are now in the process of being worked up. These data are extremely complex and will not be described until the spectra have been studied in greater detail. In a search for carbohydrates associated with

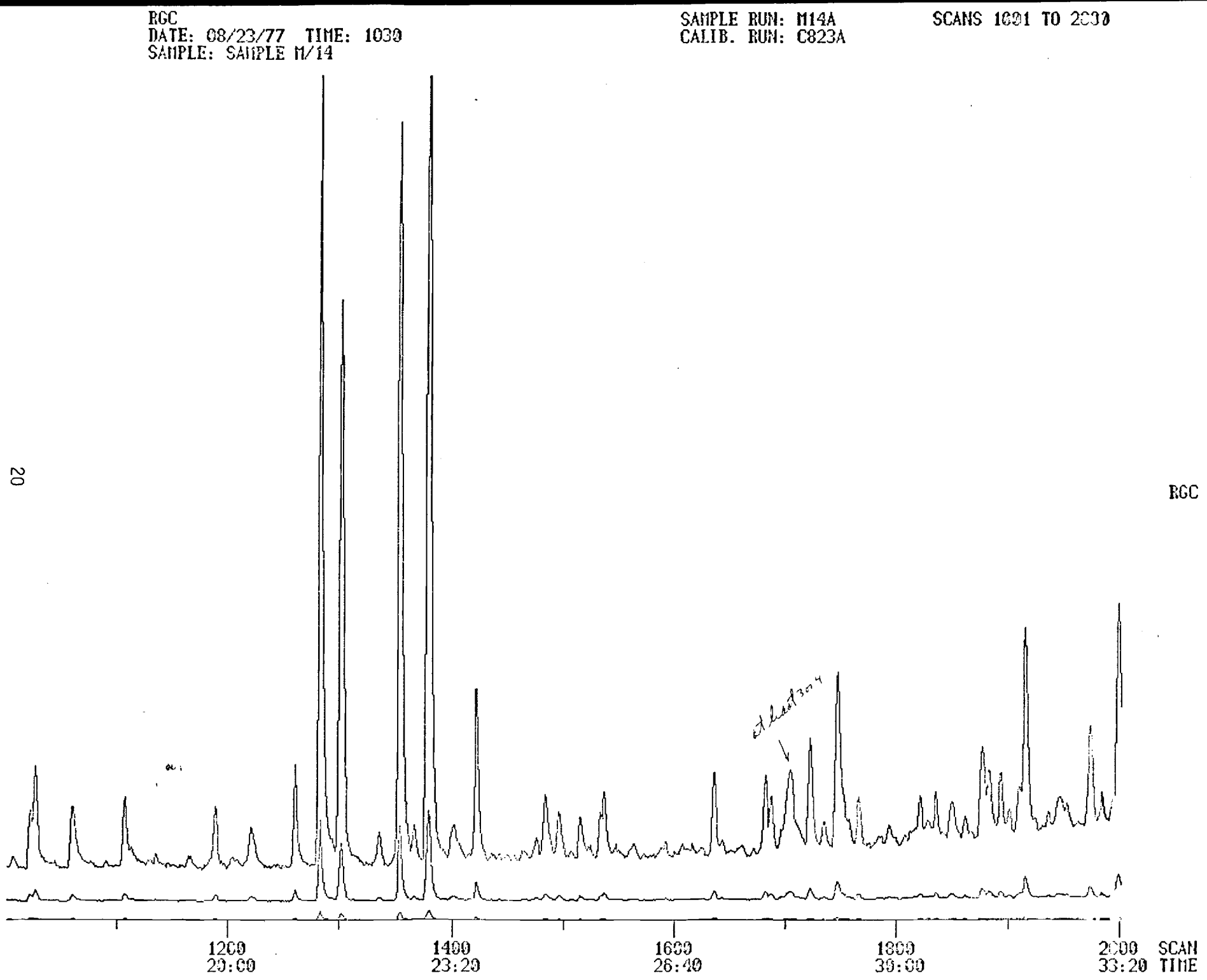
RGC
DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A SCANS 1 TO 1000
CALIB. RUN: C823A

Gas Chromatographic Separation of Methylated Oxidation Products from Humic Acid Fraction II

Figure 12




RGC
DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14
SAMPLE RUN: M14A
CALIB. RUN: C823A
SCANS 1001 TO 2000
20
RGC
1200
20:00
1400
23:20
1600
26:40
1800
30:00
2000
33:20
SCAN
TIME

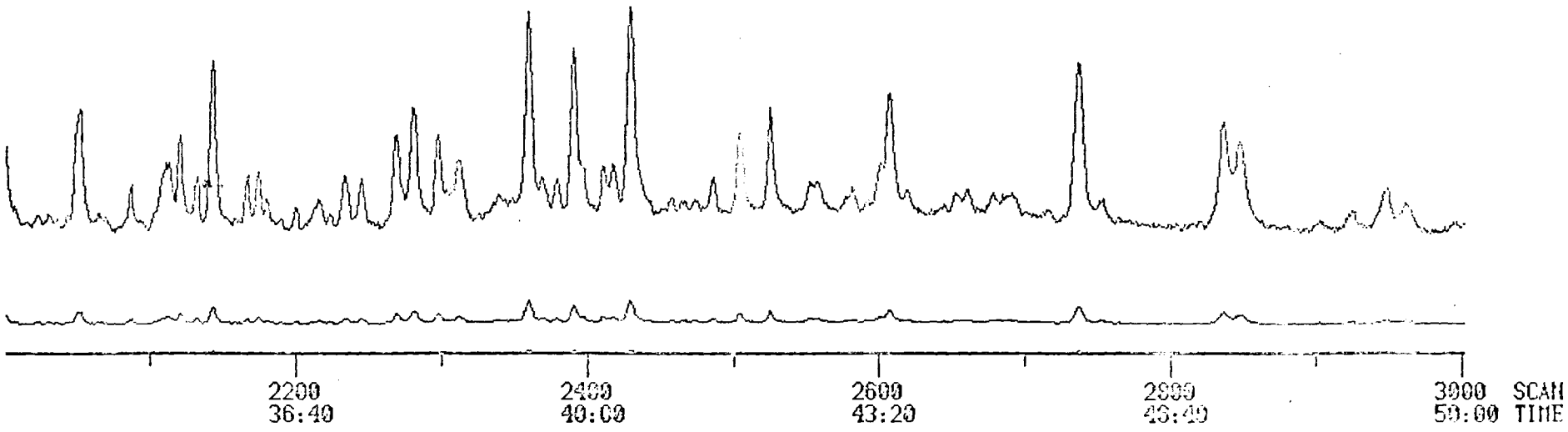
DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A
CALIB. RUN: C823A

SCANS 2001 TO 3000

21

RGC



RGC
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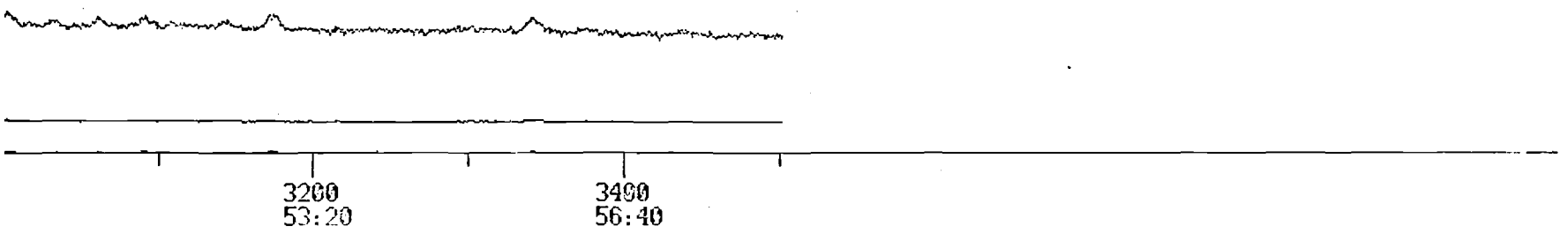
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A
CALIB. RUN: C823A

SCANS 3001 TO 3500

RGC



fraction II, we have hydrolyzed a sample with 0.2 N HCl in dioxane: water (9:1) by refluxing 4 hrs under N_2. The reaction mixture was brought to pH 4 with sodium bicarbonate and extracted with chloroform. The aqueous fraction was freeze dried. The resulting residue was silylated (using Pierce Chemical Trisil-Z), and analyzed for sugars by gas chromatography. No carbohydrates were detected. However, it should be pointed out that the hydrolysis procedure may have been much too mild to yield appreciable amounts of sugars for analysis. We intend to modify our analytical techniques further.

VI. SERIES I - FACTORIAL EXPERIMENT

A detailed plan describing the design of this experiment was sent to the sponsor for his comments on August 17. As a result of conversation with the sponsor, the following modifications have been incorporated into the design. The pH levels to be investigated will be arranged in an unbalanced fashion rather than balancing around neutrality as had originally been planned. The levels to be studied will now be 6.0, 7.5 and 9. This will encompass virtually the full range of treatment conditions and will hopefully still be a wide enough range so that differences in product distributions can still be seen. In keeping with the sponsor's suggestion that ozone not be overemphasized, the full factorial experiment will be conducted only with Welsbach ozone and mercury lamp photozone. "Ozone" from other sources will be investigated only in a more general fashion. In this way, gross differences in their chemical behavior can still be examined without an over-commitment of resources.

It will be noted that each reagent will require the running of 36 separate experiment if only two runs are put in each cell. Since it will cut our work in half if we can get by with one run per cell, a study on reproducibility would seem to be in order. This is now being conducted as follows:

The following amounts of coniferyl alcohol (Pfaltz and Bauer lot H 13760) were weighed out into a series of pre-cleaned vials:

# 1	2.1 mg	8	5.0 mg
2	2.1 mg	9	5.1 mg
3	2.0 mg		
4	2.1 mg		

The samples were treated with 10 moles of chlorine for 1 hour at pH 6.4 and ambient temperature. Residual chlorine was destroyed with Na_2SO_3 (pre-rinsed with high purity pentane) and the samples were buffered to pH 10 with Na_2CO_3-$NaHCO_3$ (pre-rinsed). Each sample was then extracted with high purity pentane (3 x 50 ml) to remove haloforms and other non-acidic organic reaction products. The aqueous layers were acidified to pH 1 with sulfuric acid prior to extraction with ether-benzene (distilled in glass) for 24 hours. In this way, the acids and phenols were removed from the reaction mixture. The pentane and ether-benzene extracts were dried and concentrated separately using the Kuderna-Danish apparatus. The concentrated pentane extracts were analyzed directly by gas chromatography. The ether-benzene extracts were treated with diazoethane prior to analysis. The analyses of the extracts are now in progress.

An unfortunate accident with the blank has greatly reduced the amount of useful information which can be obtained from this experiment. Therefore it is our intention to repeat the experiment with duplicate blanks and, as a control, a sample of unreacted coniferyl alcohol.

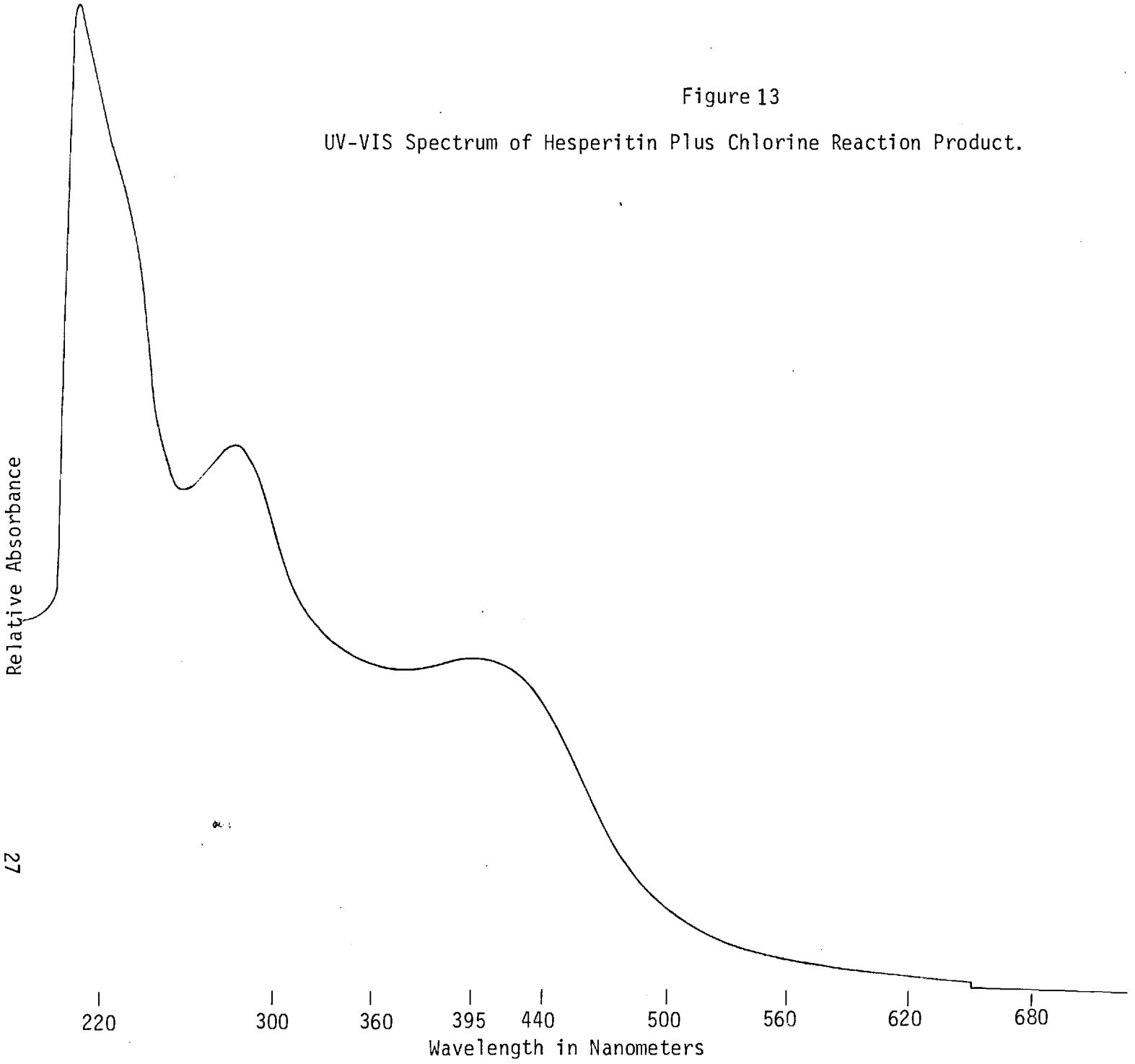
V. MODEL COMPOUND STUDIES

Hesperitin was reacted with chlorine, chlorine dioxide and photochemically generated ozone (mercury lamp) under a variety of conditions which will be described below. Each of the reaction mixtures and accompanying blanks was extracted according to the general procedure discussed in the following paragraphs. A specific case will be used to describe the extraction procedure. Subsequent paragraphs will deal only with the reaction conditions and results.

A solution of hesperitin (10 mg/l) was treated with four moles of chlorine at pH 10 for 90 minutes at ambient temperature. During the reaction period, a deep yellow color developed. The resulting reaction mixture was treated with excess sodium sulfite to remove unreacted chlorine and acidified to pH 3 with 3N sulfuric acid. The acidified solution was extracted with three 50 ml portions of distilled-in-glass pentane. In this case, the intensity of color in the aqueous layer was reduced, the pentane layer remained colorless and a yellow resinous material deposited on the walls of the separatory funnel. This yellow amorphous gum was removed from the funnel by dissolution in 95% ethanol. An ultraviolet-visible spectrum of this material was recorded and is displayed in Figure 13. The combined pentane extracts were then washed with one 50 ml portion of deionized water and extracted with two 40 ml portions of 1% aqueous sodium bicarbonate solution in order to selectively remove carboxylic acids. The sodium bicarbonate extracts were acidified with 3N sulfuric acid to pH 1 and extracted again with two 25 ml portions of ethyl ether (freshly washed with acidified ferrous sulfate solution to remove peroxides). The ethereal solution was treated at ice-water temperatures with an ethereal diazoethane solution [prepared by co-distillation of ether and diazoethane formed by reaction of 5N sodium hydroxide (6 ml) and N-ethyl-N-nitroso-N'-nitroguanidine (0.72 g) dissolved

Figure 13

UV-VIS Spectrum of Hesperitin Plus Chlorine Reaction Product.



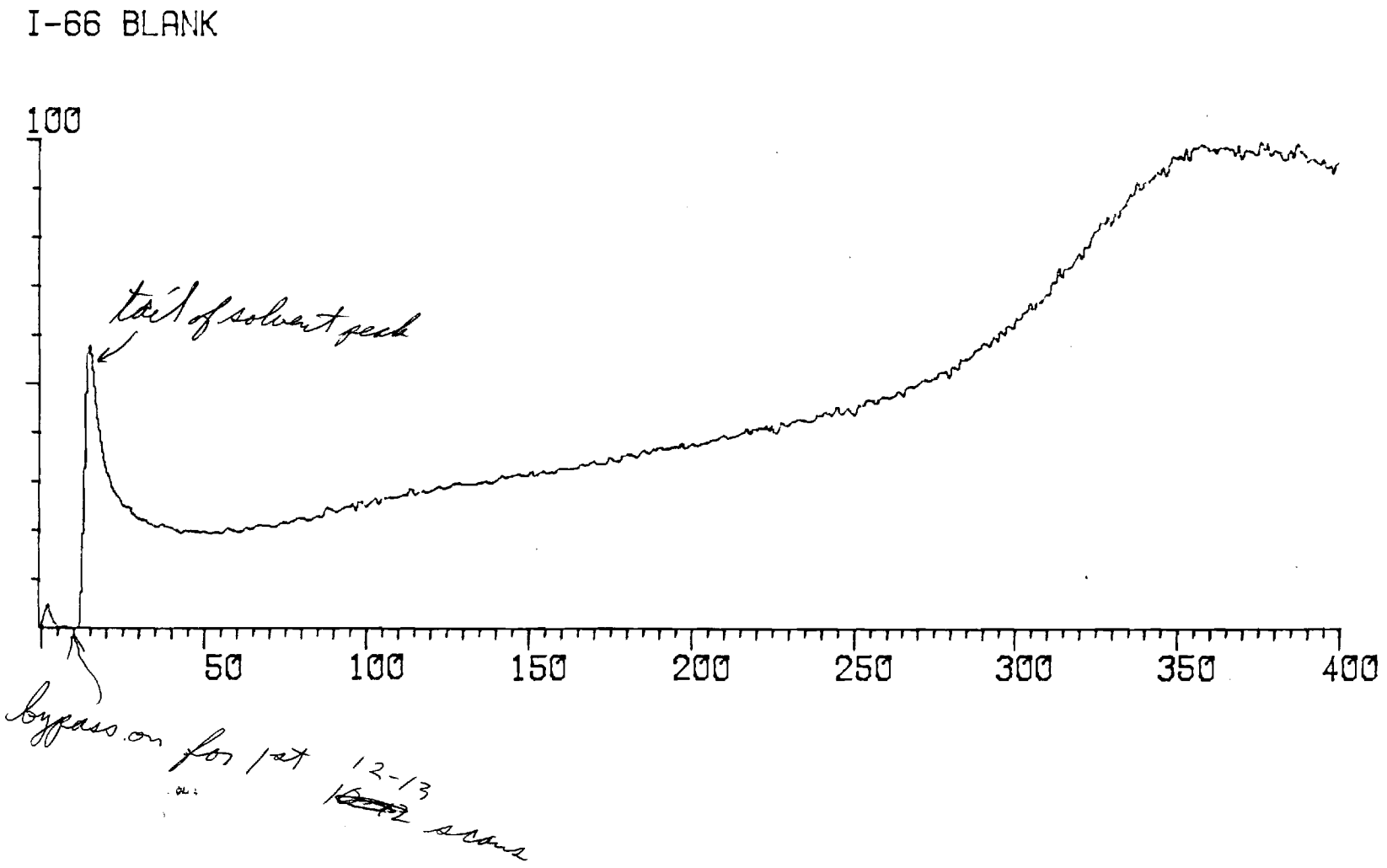
in ether (125 ml)] until a yellow color persisted for 2-3 minutes. The resulting solution of ethyl esters were allowed to warm to room temperature in a hood, dried over granular, anhydrous, sodium sulfate and concentrated to a volume of two ml in a Kuderna-Danish apparatus. The original pentane extract after sodium bicarbonate extraction was extracted again with two 40 ml portions of 1N sodium hydroxide solution in order to isolate phenolic compounds. The pentane solution from which the phenols and acids had been removed was concentrated to a volume of two ml in a Kuderna-Danish apparatus. The sodium hydroxide extracts were quickly re-acidified and re-extracted with fresh ethyl ether (2x40 ml).

Six such mixtures of ethyl esters from the carboxylic acid fractions resulting from treatment of hesperetin with chlorine, chlorine dioxide and photozone were analyzed on the Finnigan GL/MS system at their California laboratories. We are pleased to inform the sponsor that the expense of this trip was largely underwritten by the Finnigan Corporation as a means of compensating for their inability to deliver our instrument during the month of August.

The total ion current mass chromatogram of the solvent-reagent blank from the isolation and ehtylation procedure was measured. The plot of total ion current vs scan number (directly proportional to time of elution) from the GC unit (Figure 14) showed a response peak during the first thirty scans (arbitrary time units), a slow response rise for the period between scan number 30 and scan number 300, and a response maximum at scan number 360. A specific (AMU 149) ion monitoring of the blank gave a response similar to the total ion current response except for the lack of a peak between scan number 10 and scan number 45. A copy of this plot is presented in Figure 15.

Figure 14

Total Ion Monitoring - Reagent Blank for Derivatized Acid Fractions



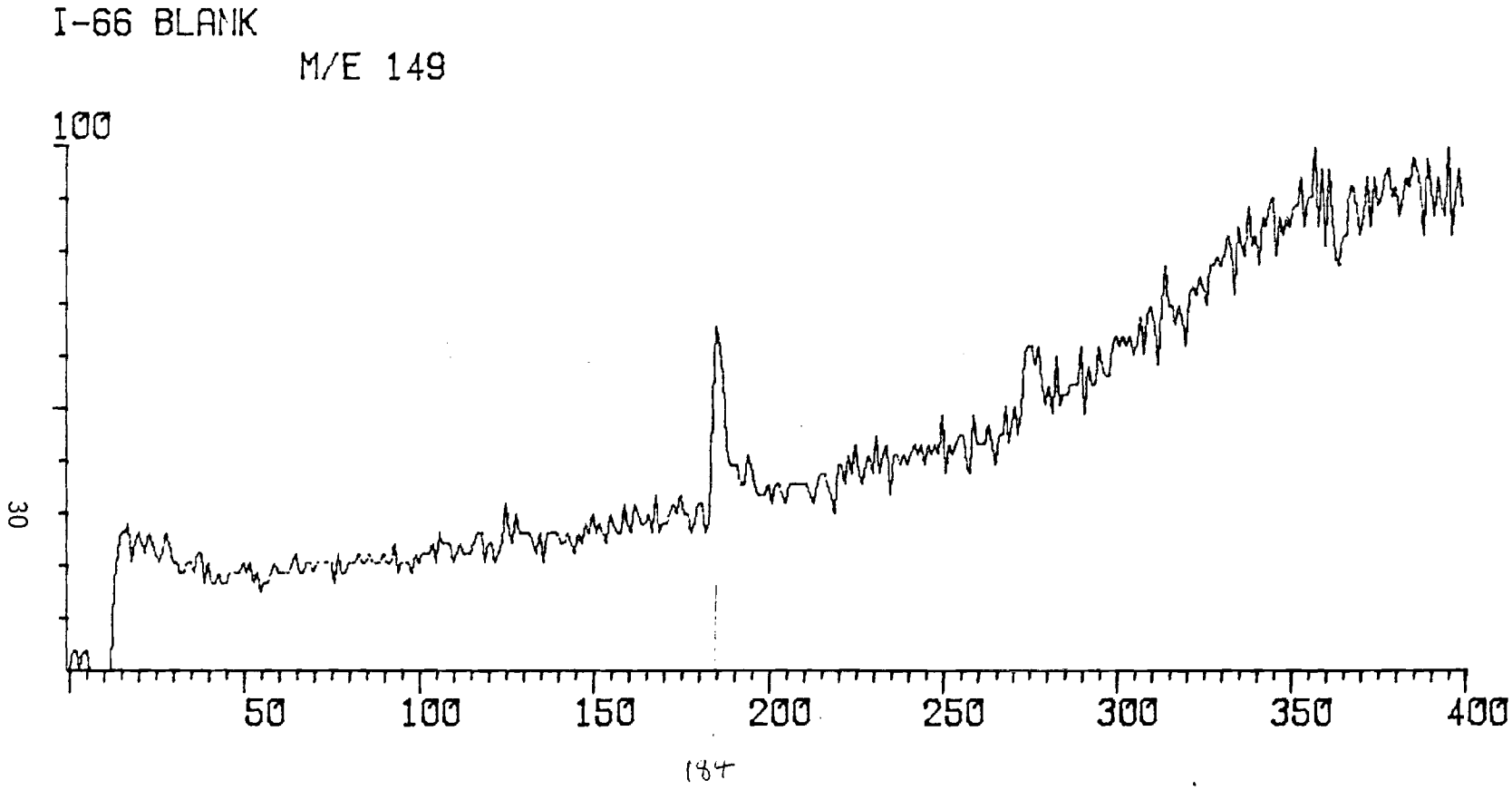


Figure 15

Specific Ion Monitoring at AMU 149 - Reagent Blank for Derivatized Acid Fractions.

The derivatized acid fraction from the previously described reaction of hesperitin with four moles of chlorine at pH 10 provided the total ion and specific ion chromatograms shown in Figure 16 and 17. The specific ion chromatogram shows peaks at scan numbers 115, 151, 165 and 183.

The most intense fragmentation pattern was obtained from materials eluting at scan number 199 in the total ion chromatogram. Accordingly a detailed analysis of this fragmentation pattern is being attempted as a first step in elucidating the structures of as many of these materials as possible.

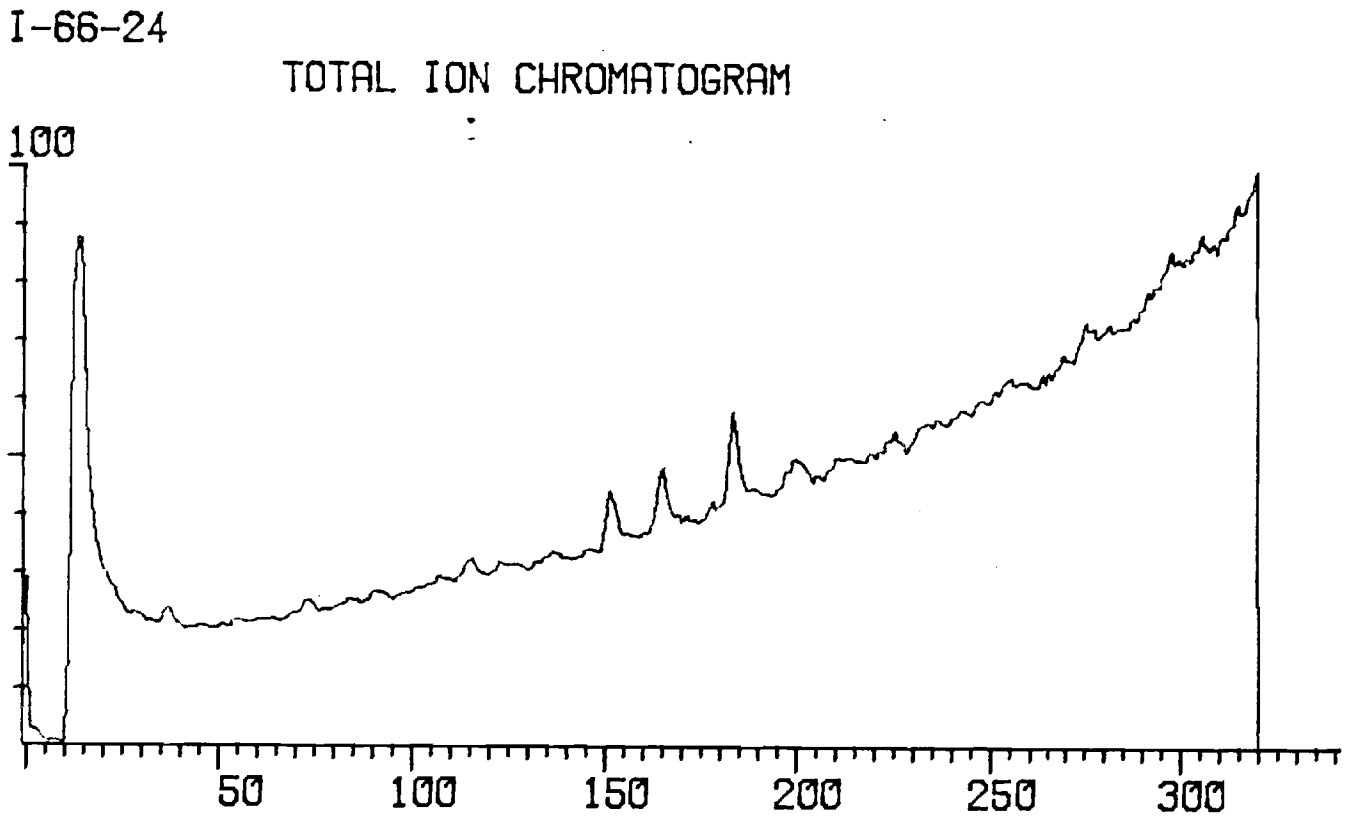


Figure 16

Total Ion Chromatograms for Derivatized Acid Fractions from Hesperitin.

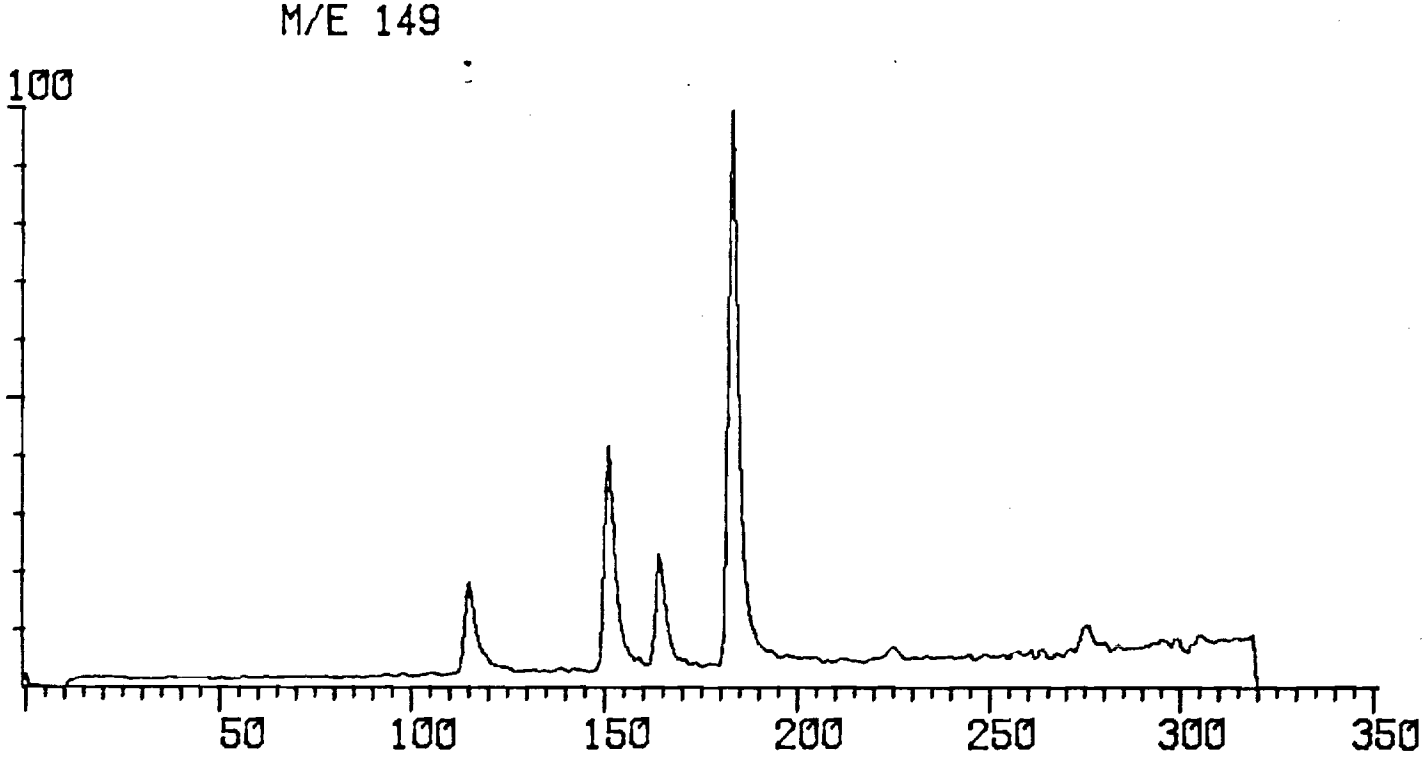


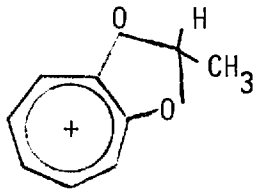
Figure 17

Specific Ion Chromatogram for Derivatized Acid Fraction from Hesperitin.

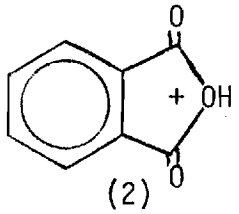
However, the data have been in our hands for only a few days and as yet we are not confident that we have a structure which is consistent both with the expected course of the oxidation reaction and with the observed mass spectral fragmentation pattern. (See Figure 18.)

In the course of agonizing over the interpretation of this spectrum and other spectra, it has come to our attention that the problems to be faced in the interpretation of such data are truly staggering. For example, reactive acids such as can be expected to be found in the fractions under discussion might recombine under the conditions of isolation and treatment with diazoethane, thus leading to structures having a different arrangement of functional groups than would otherwise be expected.

Contamination will have to be more strictly avoided. The presence of a strong m/e 149 ion in many of the scans from the acid fraction might be due to a rearrangement ion having the formula $C_9H_9O_2$ for which structure (1) can be postulated or the plasticizer derived ion having postulated structure (2).



(1)



(2)

If precise mass measurements indicate that $C_8H_5O_3$ is the dominant ion in many of the constituents of the m/e 149 mass chromatogram, we shall have to root out and eliminate the source of such extraneous materials.

Other reaction mixtures for which we have similar raw mass spectral data include the products resulting from treating the 10 mg/l solution of

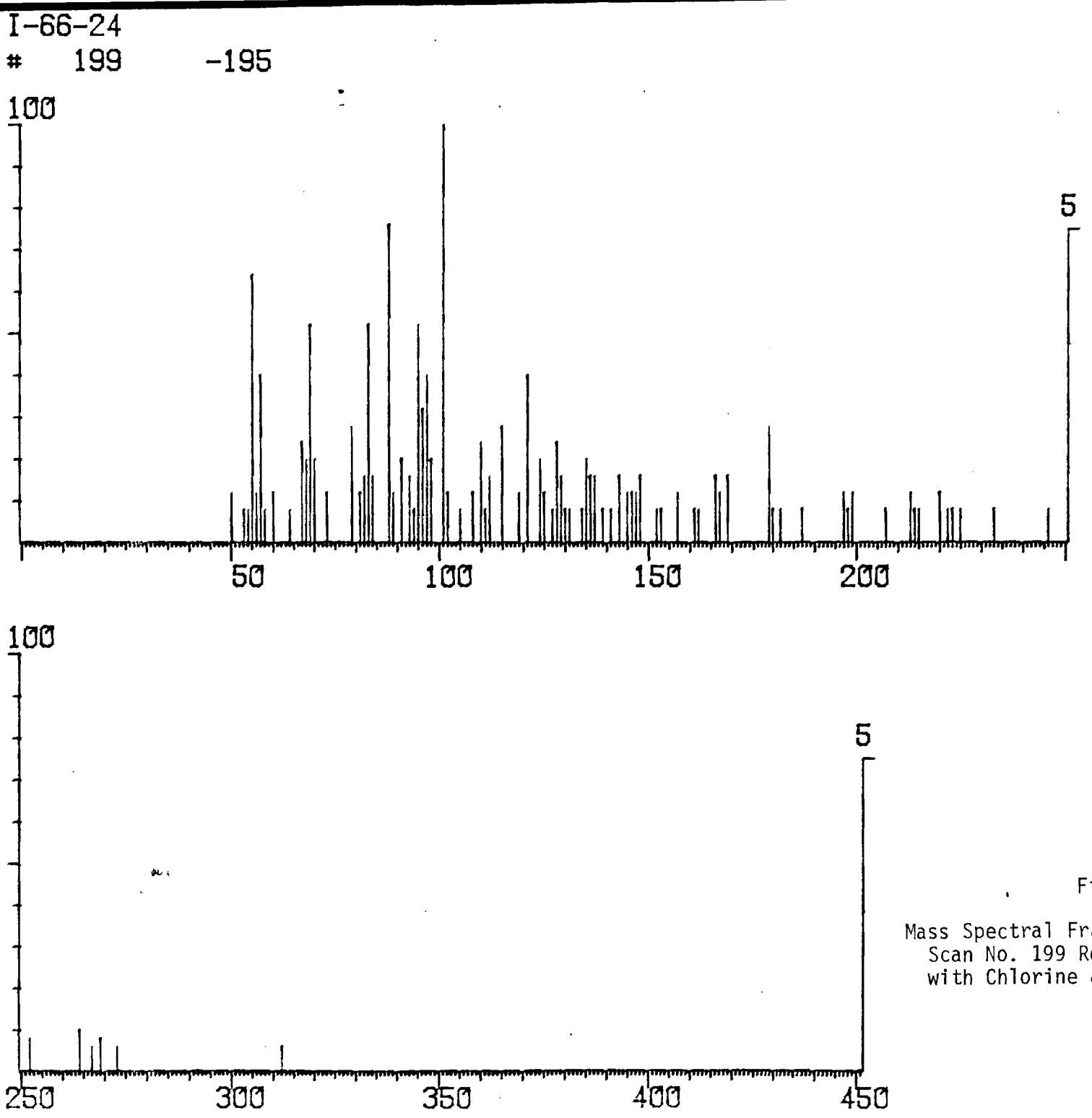


Figure 18

Mass Spectral Fragmentation Pattern - Scan No. 199 Reaction of Hesperitin with Chlorine at pH 10.

hesperitin with chlorine dioxide and photozone for 90 minutes at room temperature and pH 10. The reaction products resulting from the treatment of hesperitin (9.3 mg/l) with chlorine, chlorine dioxide and photozone at pH 7.5 and ambient temperatures (see previous monthly report page 11) are still under study.

Additional uninterpreted mass spectral data are available for the methylated oxidative degradation products described in Section IV. Data is also available for silylated extracts of the methylated humic substances described in the same section and for a silylated carbohydrate standard containing xylose, galactose and ribose. At this time, the interpretation is in such a preliminary stage that it would seem best to withhold conclusions until the evidence can be more carefully weighed.

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

October 4, 1977

by

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DISCLAIMER

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I. PERSONNEL

No personnel changes have taken place during this reporting period.

II. EQUIPMENT

Our new Finnigan 4023 GC/MS system is now in operation. We are continuing with our round-the-clock efforts to familiarize ourselves with the operation of this equipment and thoroughly test its capabilities. We have experienced more than the usual number of start-up problems, but have put them behind us at a faster-than-usual pace due mainly to extra effort on our part and on Finnigan's part. The EES electronic capabilities have also been very helpful in this respect.

We have run samples in the EI, CI and solids probe modes. We have tested conventional columns and capillary columns in the gas chromatograph. We have exercised the data processing capabilities of the INCOS system and are already getting useful results, particularly on our humic acid degradation studies. These results will be described more fully in later sections of this report.

The design of our mini-pilot water treatment facility is presented below for your review and comments. A continuous flow system is envisioned featuring the following component treatments:

1. Presedimentation
2. Initial pH adjustment and chemical addition
3. Mixing basin
4. Flocculation
5. Sedimentation
6. Sand/carbon filters
7. Storage
8. Final pH, residual adjustment

The scale envisioned is such that a flow rate of somewhat over 100 ml per minute will be maintained. The equipment will be sized so that the

reservoir will hold enough water (100 gal) to run for 24-48 hours. A pre-leached (hot water) polyethylene tank is our first choice of material for the reservoir. If leaching or adsorption appears to be a problem, a metal reservoir will be considered. The water to the reservoir will be supplied by our high-capacity continental water conditioning system which is equipped to remove both organic and inorganic contaminants.

A constant head device (1 liter capacity) will be employed to minimize variations in flow during the course of an experiment. The mixing basin which will serve as a site for the addition of chemicals will also be 1 liter in size. The flocculator will be designed to retain the water for 20-30 minutes (capacity 4 liters). The sedimentation basin will be of sufficient size (30-35 l) to hold the water for 3.5-4.0 hours. The sand/granular activated carbon filters will be placed in columns so that a realistic depth can be achieved without an undue exaggeration of the surface area. A storage basin of 40-50 liter capacity will be required to achieve a retention time of 4-5 hours. A final chemical injector/mixer of 1 liter capacity will be provided to simulate final pH and residual adjustment. Every effort will be made to minimize the use of contaminating materials in pumps, plumbing and containers. A schematic diagram is presented in Figure 1.

III. GAS CHROMATOGRAPHIC STUDIES

Since trihalomethane production is a legitimate aspect of this research program, we have been quite interested in applying the Lupton detector to this task. Since we are using the pentane extraction procedure described by Glaze, it was important to establish conditions which were capable of separating chloroform from pentane. This desire prompted experimentation with extremely low flow rates which coincidentally could be handled by this detector. Since capillary columns require flow rates in the five ml/min

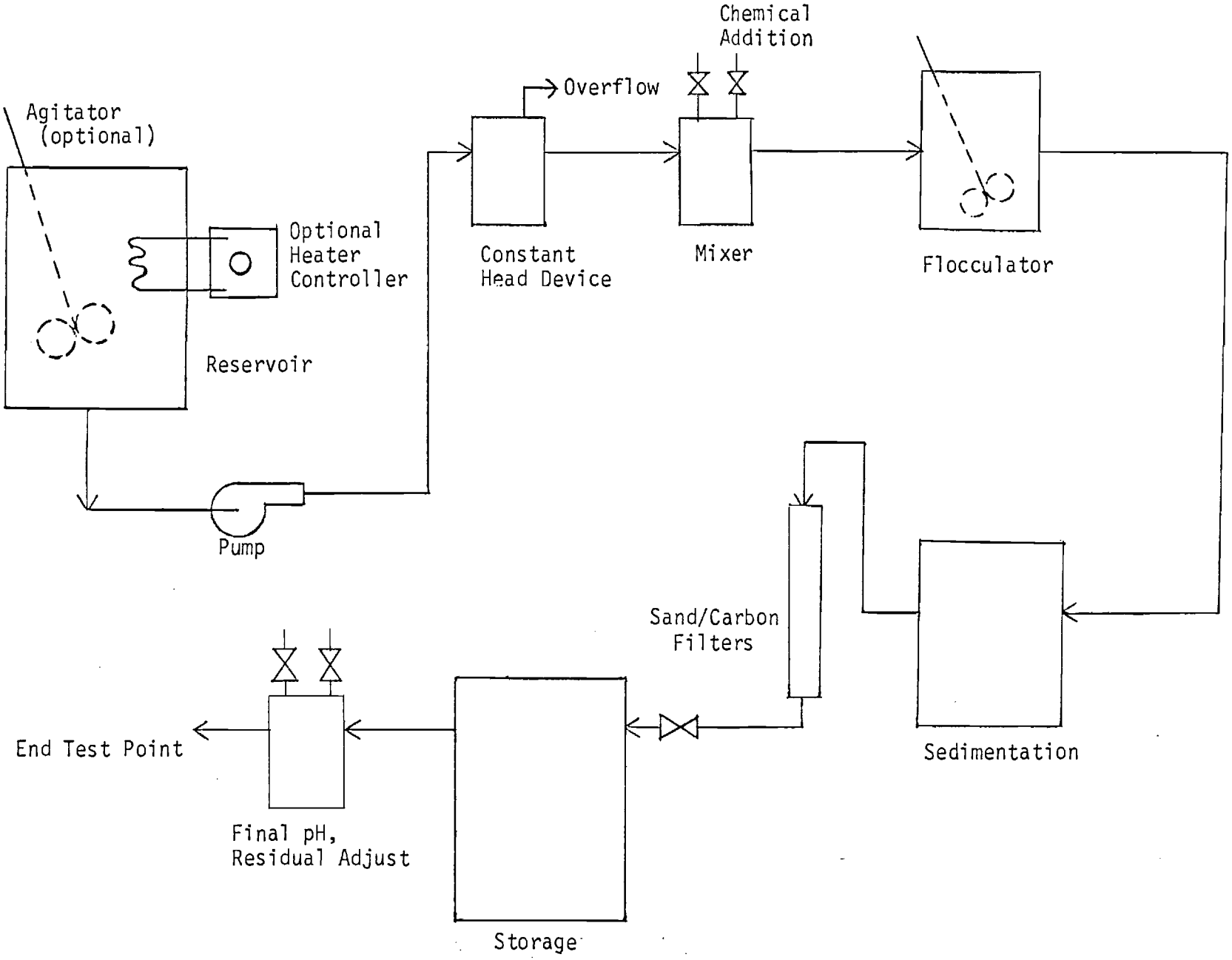


Figure 1. Schematic Diagram of Mini-Pilot Facility for Simulation of Commercial Water Treatment.

range which are considered to be below the minimum flows accepted by conventional ED detectors, we tried a flow rate of 4.5 ml/min. Figure 2 shows the results of a 1 µl injection of pentane containing 2.5×10^{-6} gram $CHCl_3$ at 4.5 ml N /min.

Several problems have been created by this low flow rate technique which will require further work. For example, there is a shift in the direction of responses which appears to be related, at least in part to the temperature of the detector.

Also the response to $CHCl_3$ is presently unacceptably low. The column being used is not the best for lot temperature operations now being used to achieve the separations. Figure 3 shows a marked increase in response as the cathode voltage is increased from 0.20 Volts to 1.0 Volt. The observed baseline drift is due in part, to a cold ($22^{o}C$) cell. It is hoped that a different column and continued efforts will produce a new and superior technique for the analysis of trihalomethanes.

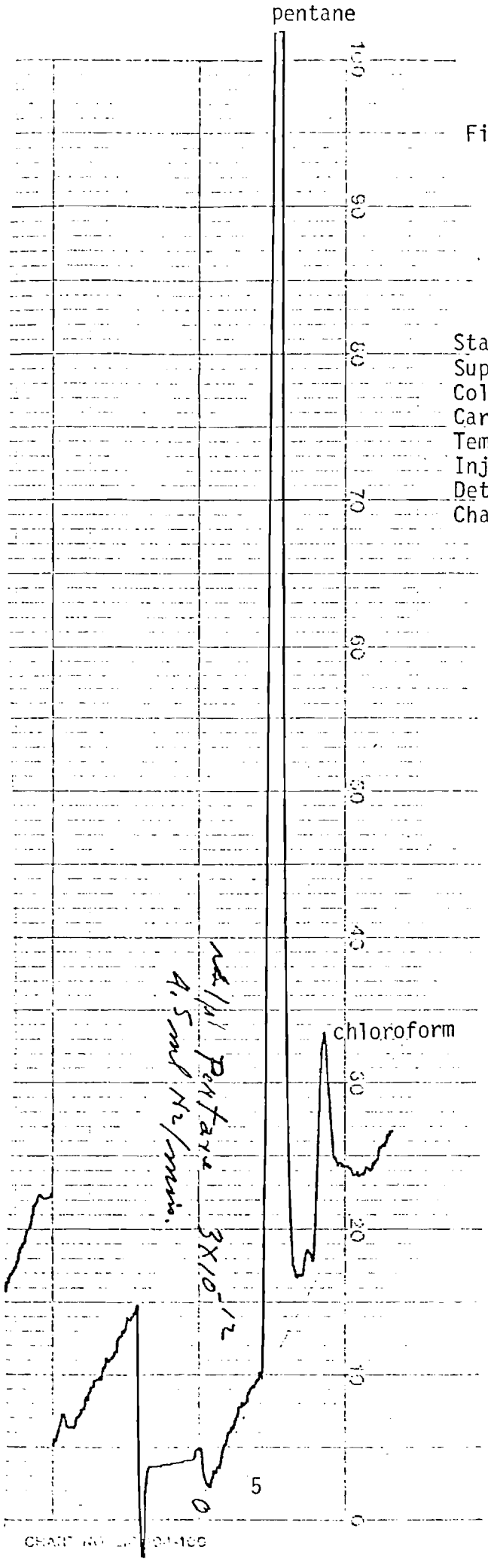


Figure 2. Separation of Chloroform and Pentane

Stationary Phase: 3% SE30
Support Chromosorb W
Column 6'x3mm
Carrier Gas N_2
Temperature $22^{o}C$
Injector $75^{o}C$
Detector $22^{o}C$
Chart 16"/hr.

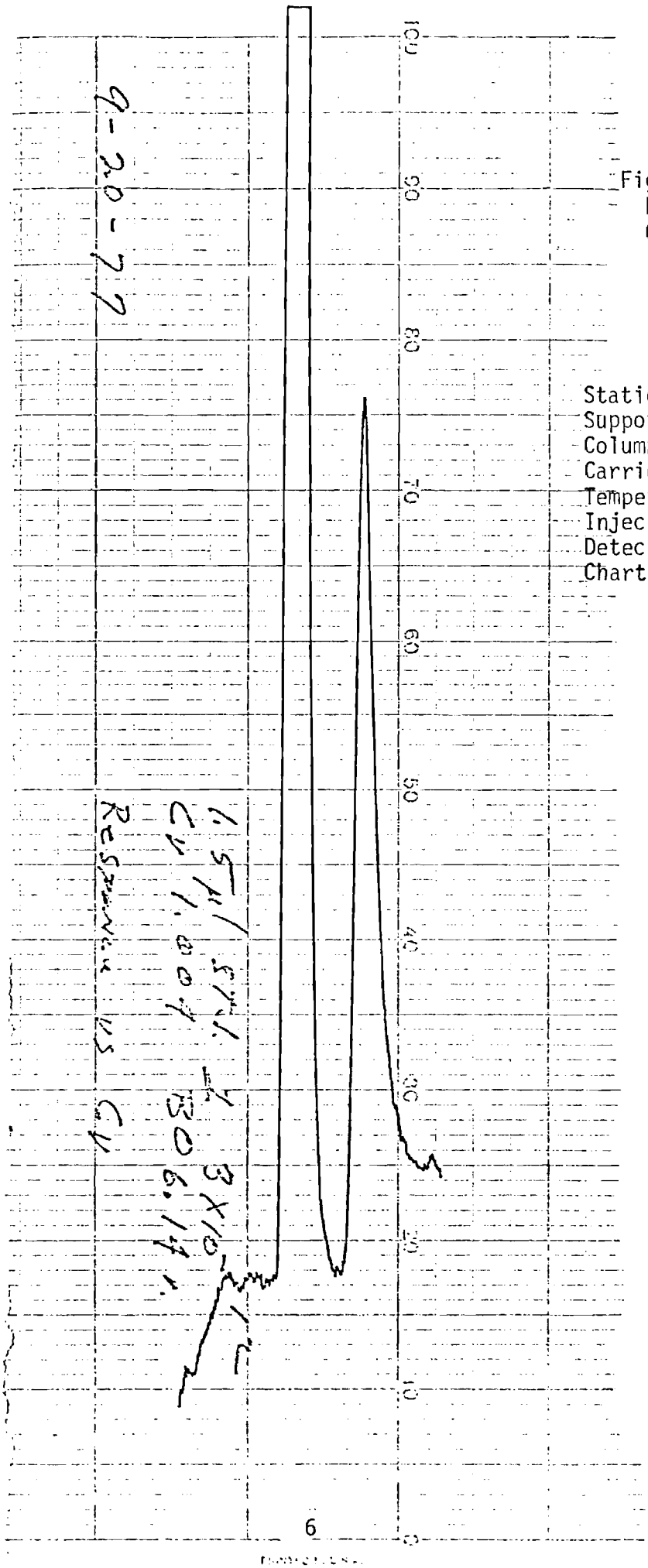


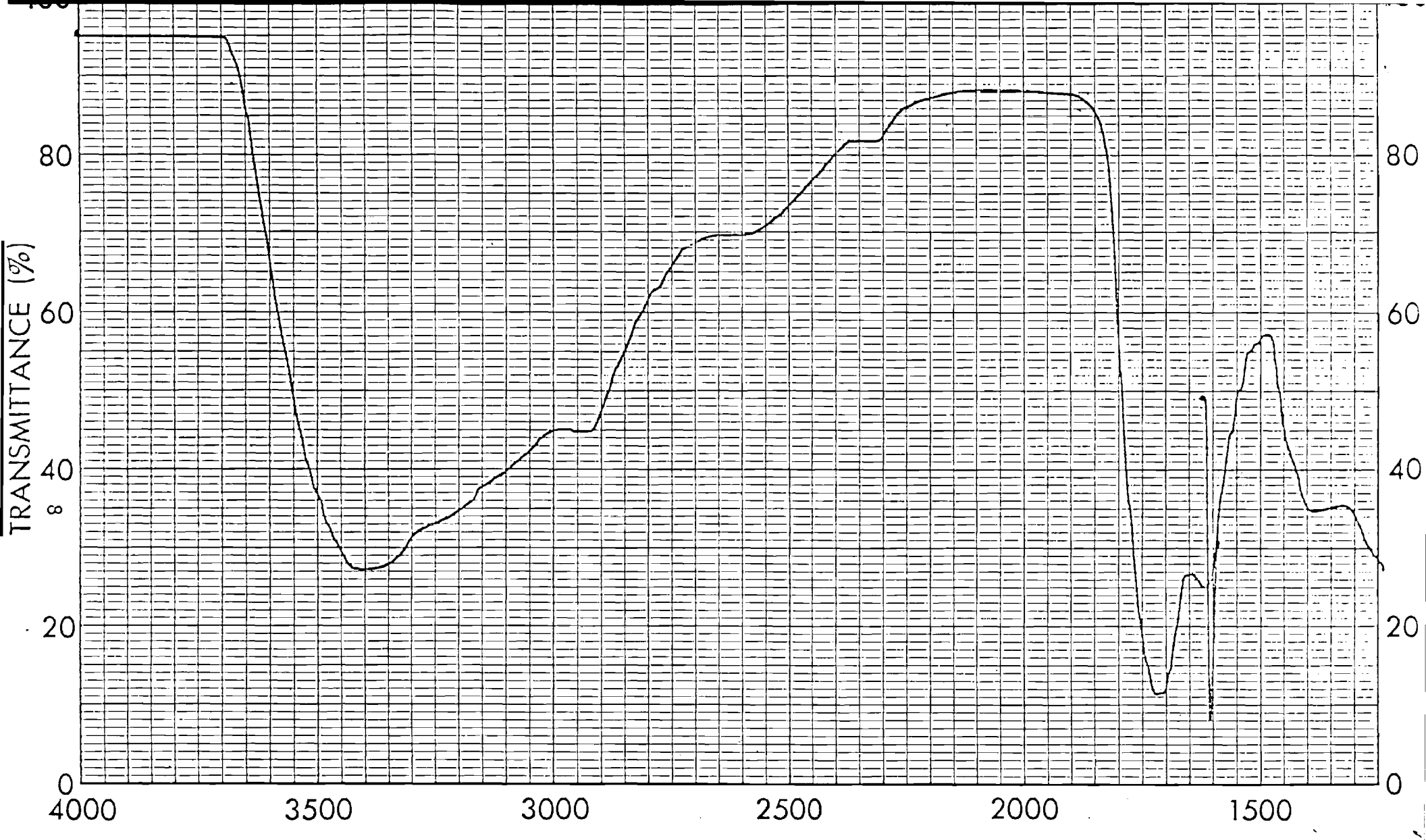
Figure 3. Increased Response with Increased Cathode Voltage (compare with Figure 2)

Stationary Phase 3% SE30
Support Chromosorb W
Column 6'x3mm
Carrier Gas N_2
Temperature 22°C
Injector 75°C
Detector 75°C
Chart 16"/min.

IV. SAMPLES AND PRELIMINARY SEPARATIONS

A sample of aquatic humic material was subjected to sequential treatment with diazomethane and acetic anhydride in pyridine in an effort to gain some insights regarding the nature of the hydroxyl groups present in humic materials. The infrared spectrophotometer was employed before and after each treatment as a means of determining the fate of the OH functions. As can be seen in Figure 4, a very large O-H band extends from 3600 to 2300 cm^{-1} in the untreated material. This is indicative of carboxylic acid and other strongly hydrogen-bonded functions. Other -OH functions may also be present, but would be obscured by the broad and intense absorption in this region. Following methylation with diazomethane and sample drying, the absorption in this region is greatly reduced (but not eliminated) thus indicating the presence of some aliphatic -OH groups (see Figure 5). The methylated humic material was subsequently acetylated, a treatment which resulted in the complete elimination of the -OH absorption (see Figure 6). Thus it can be concluded that aquatic humic substances have aliphatic hydroxyl groups. It can further be concluded that these groups are primary and or/secondary in nature since the last treatment is not sufficiently rigorous to acetylate tertiary -OH functions.

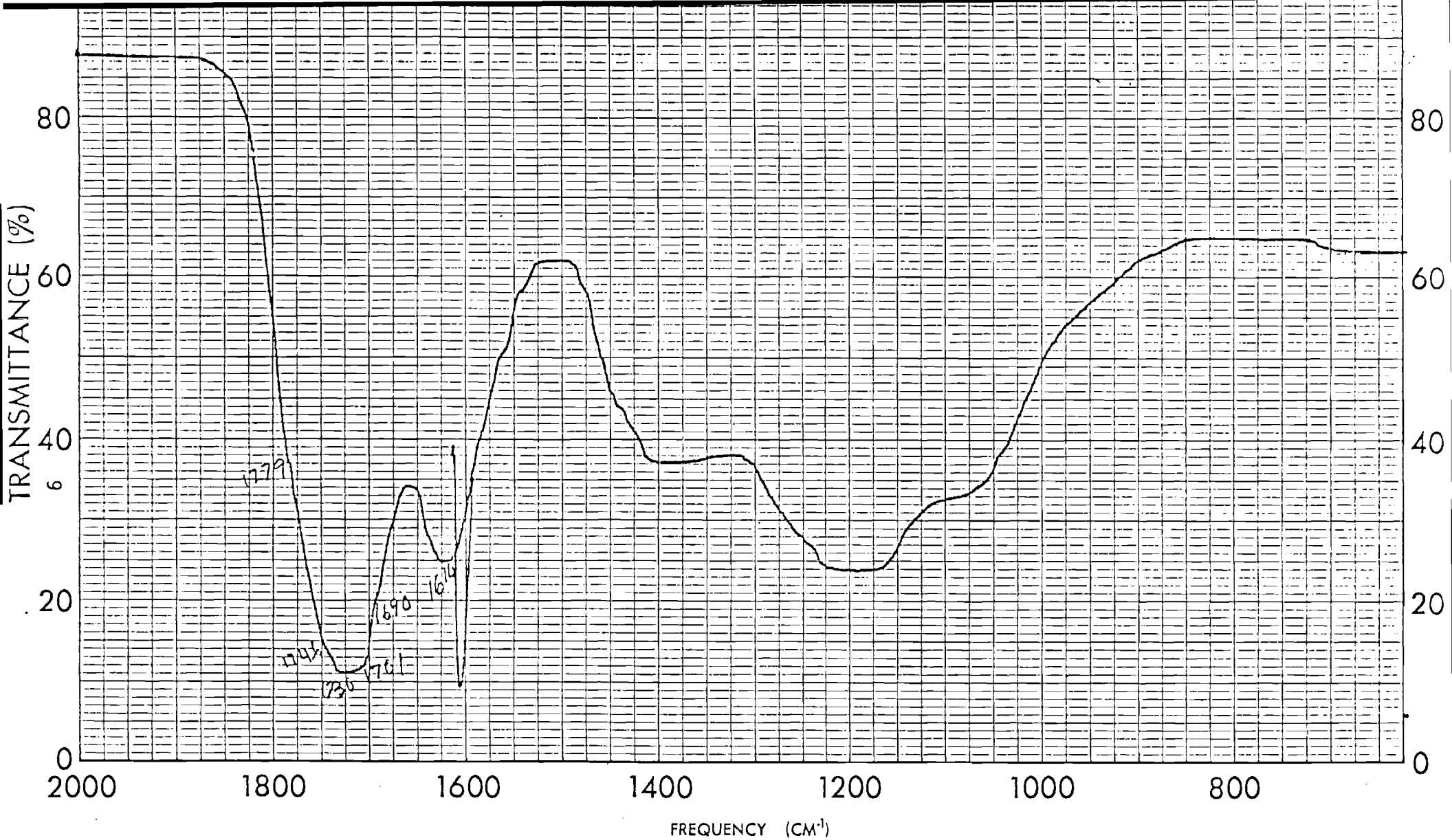
Aquatic humic substances (0.8 g) were methylated with diazomethane in the usual way. The yield, after filtration to remove insoluble material was 0.6 g. This material was subsequently dissolved in 50 ml of methylene chloride, cooled to -78^{o} in a dry ice-acetone bath and ozonized for 1 hour. The reaction mixture was then treated with water, zinc and acetic acid in order to decompose the ozonides. Gas chromatographic separation and identification is in progress.



SAMPLE RS36A	CURVE NO. Figure 4	SCAN SPEED	OPERATOR M. Gauthier
	CONC. Aquatic Humic Substances	SLIT	DATE 7-13-77
ORIGIN	CELL PATH (no treatment)	REMARKS	
SOLVENT KBr.	REFERENCE		

PART NO. 237-1034

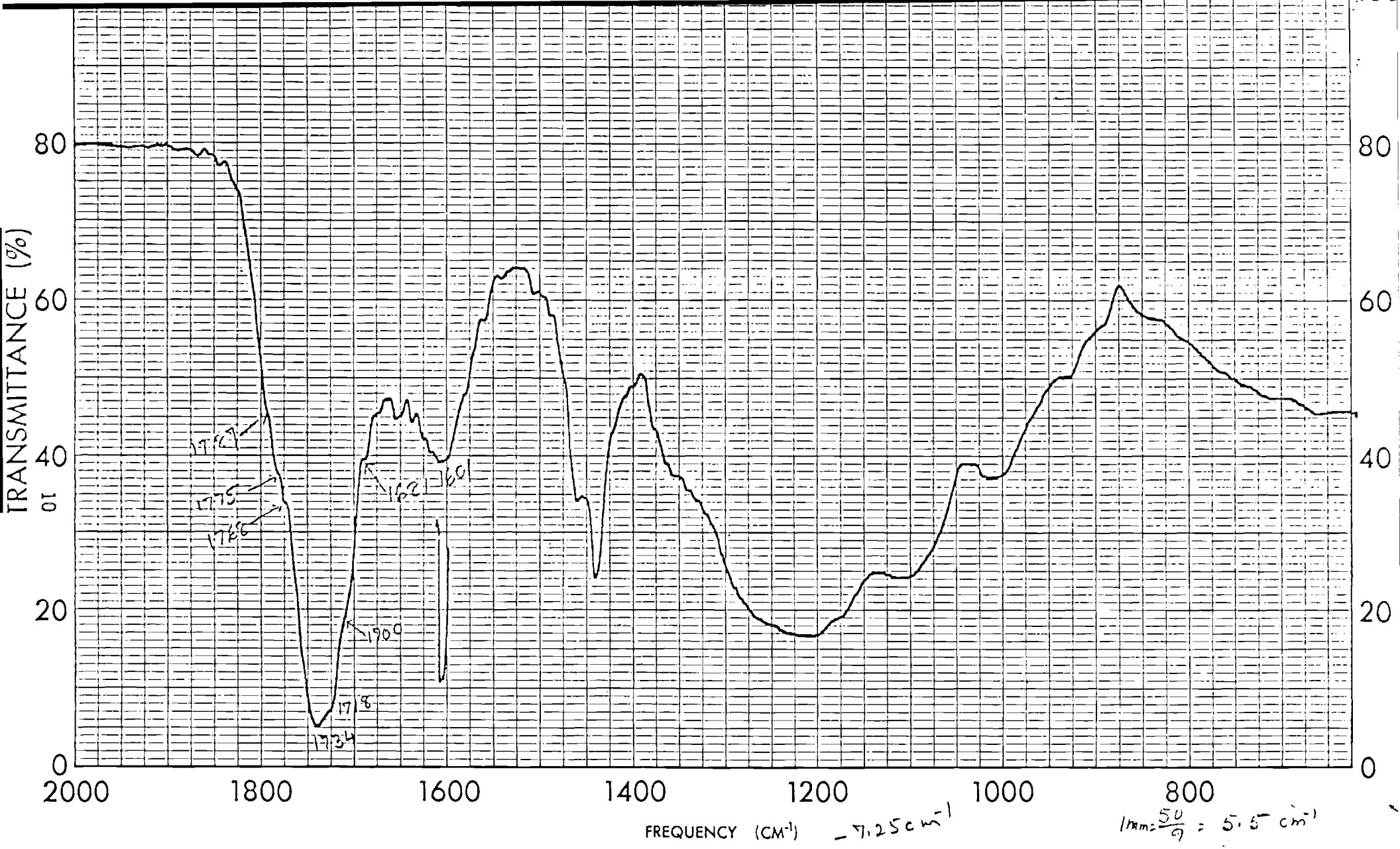
PERKIN-ELMER



SAMPLE ~~A-4 aq.~~ RS36A	CURVE NO. Figure 4 (continued)	SCAN SPEED	OPERATOR M. Ghosal
	CONC. Aquatic Humic Substances	SLIT	DATE 7-13-77
ORIGIN	CELL PATH (no treatment)	REMARKS	
SOLVENT KBr	REFERENCE		

PART NO. 237-1035

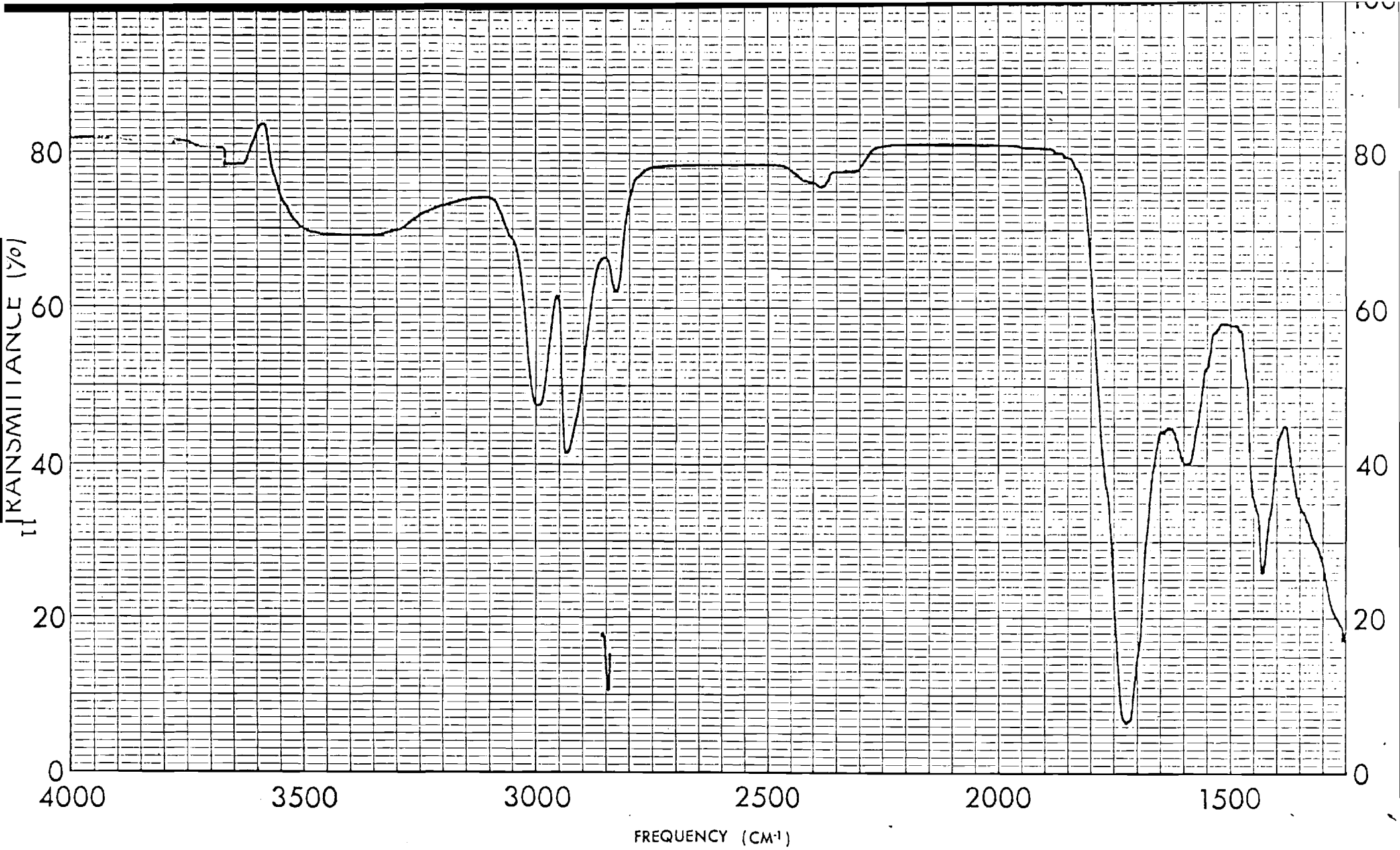
PERKIN-ELMER



SAMPLE PS 36 A. Methylated (Desalted)	CURVE NO. Figure 5 (continued)	SCAN SPEED	OPERATOR
	CONC. Methylated Humic Substances	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT $CHCl_3$	REFERENCE		

PART NO. 237-1035

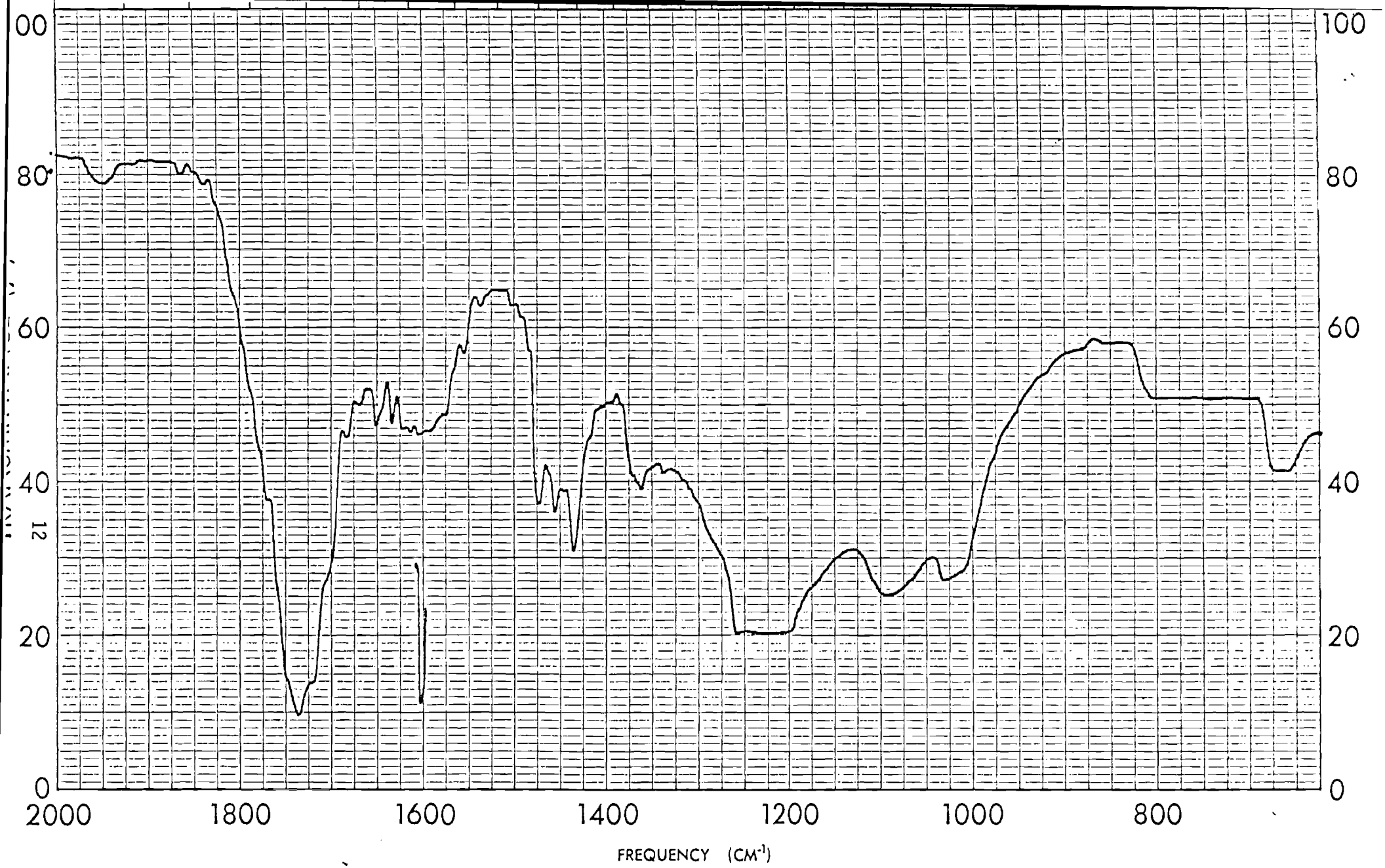
PERKIN-ELMER



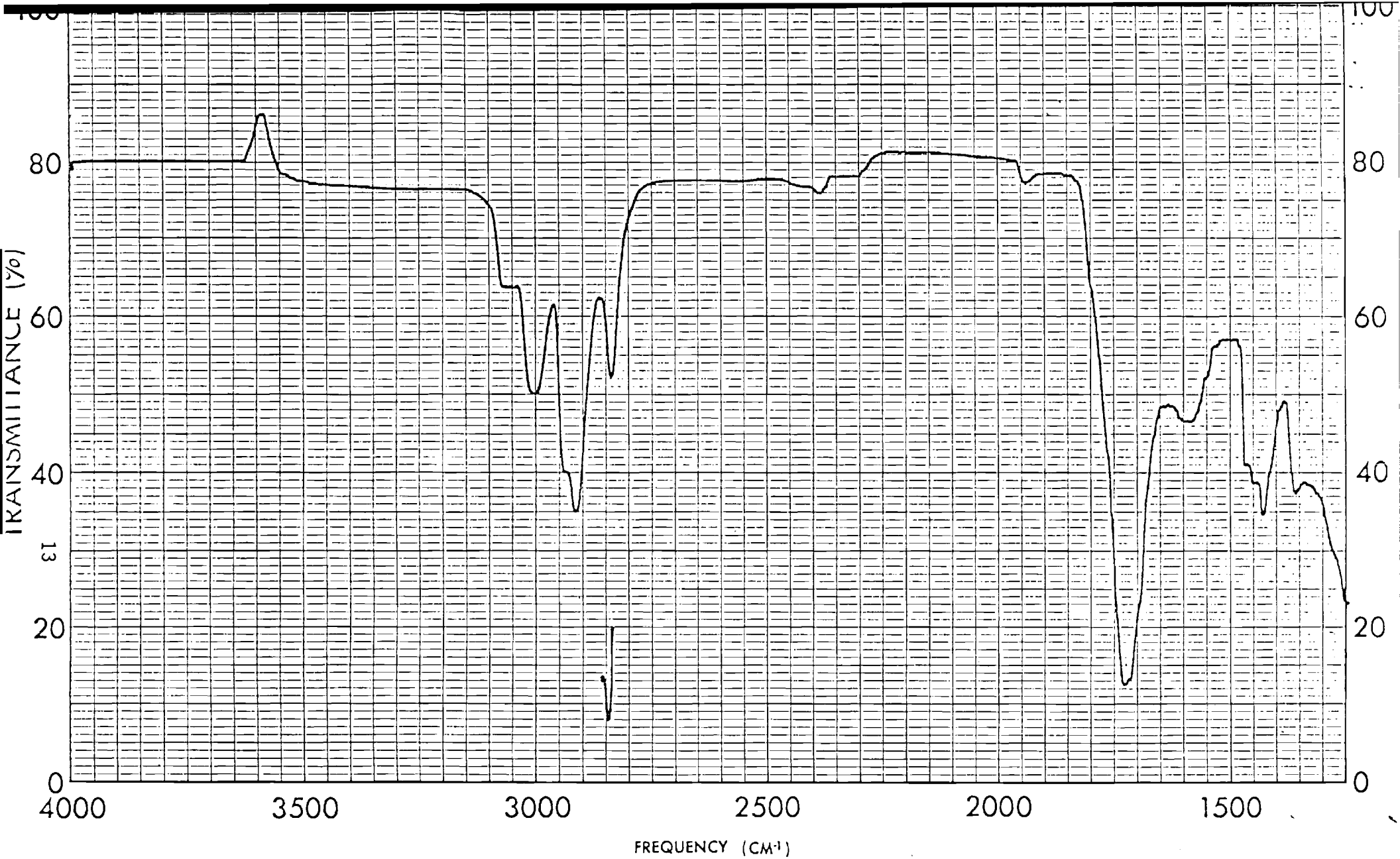
SAMPLE RS 36A methylated (desalted)	CURVE NO. Figure 5 (continued)	SCAN SPEED	OPERATOR
	CONC. Methylated Humic Substances	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT $CHCl_3$	REFERENCE		

PART NO. 237-1034

PERKIN-ELMER



SAMPLE P.S.2.A methylated — acetylated	CURVE NO. Figure 6 CONC. Acetylated, Methylated Aquatic	SCAN SPEED SLIT	OPERATOR DATE
ORIGIN	CELL PATH Humic Substances	REMARKS	
SOLVENT	REFERENCE		



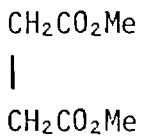
SAMPLE RSFA Chem Product - Acetylated	CURVE NO. Figure 6 (continued)	SCAN SPEED	OPERATOR
	CONC. Acetylated, Methylated Aquatic	SLIT	DATE
ORIGIN	CELL PATH Humic Substances	REMARKS	
SOLVENT CHCl3	REFERENCE		

PART NO. 237-1034

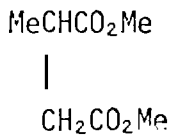
PERKIN-ELMER

Another sample of aquatic humic substances (0.1 g) was dissolved in water, brought to pH8 with dilute sodium hydroxide and reacted with excess sodium borohydride in an effort to selectively reduce the aldehyde, ketone or quinone functions which might have been present in the original material. The resulting product was acidified with acetic acid, freeze dried and subsequently methylated with diazomethane in a methanol. The reaction mixture which was insoluble in methanol, remained insoluble when transferred to chloroform for reaction with acetic anhydride in pyridine. The resulting product retained its insolubility indicating that undesired side reactions may have occurred. Work is continuing in this area.

Teh reaction products from an earlier oxidative degradation with aqueous permanaganate have been separated and analyzed by the methods of capillary gas chromatography/mass spectrometry (see September 2 Progress Report page 18, 19). The processing of this data is not proceeding using our new INCOS system softward. A number of tentative identifications have already been made which are summarized below. The numbers given with the structures refer to positions in the chromatogram which is presented in Figure 7.



2) Succinic acid dimethyl ester



3) 2-Methyl succinic acid dimethyl ester

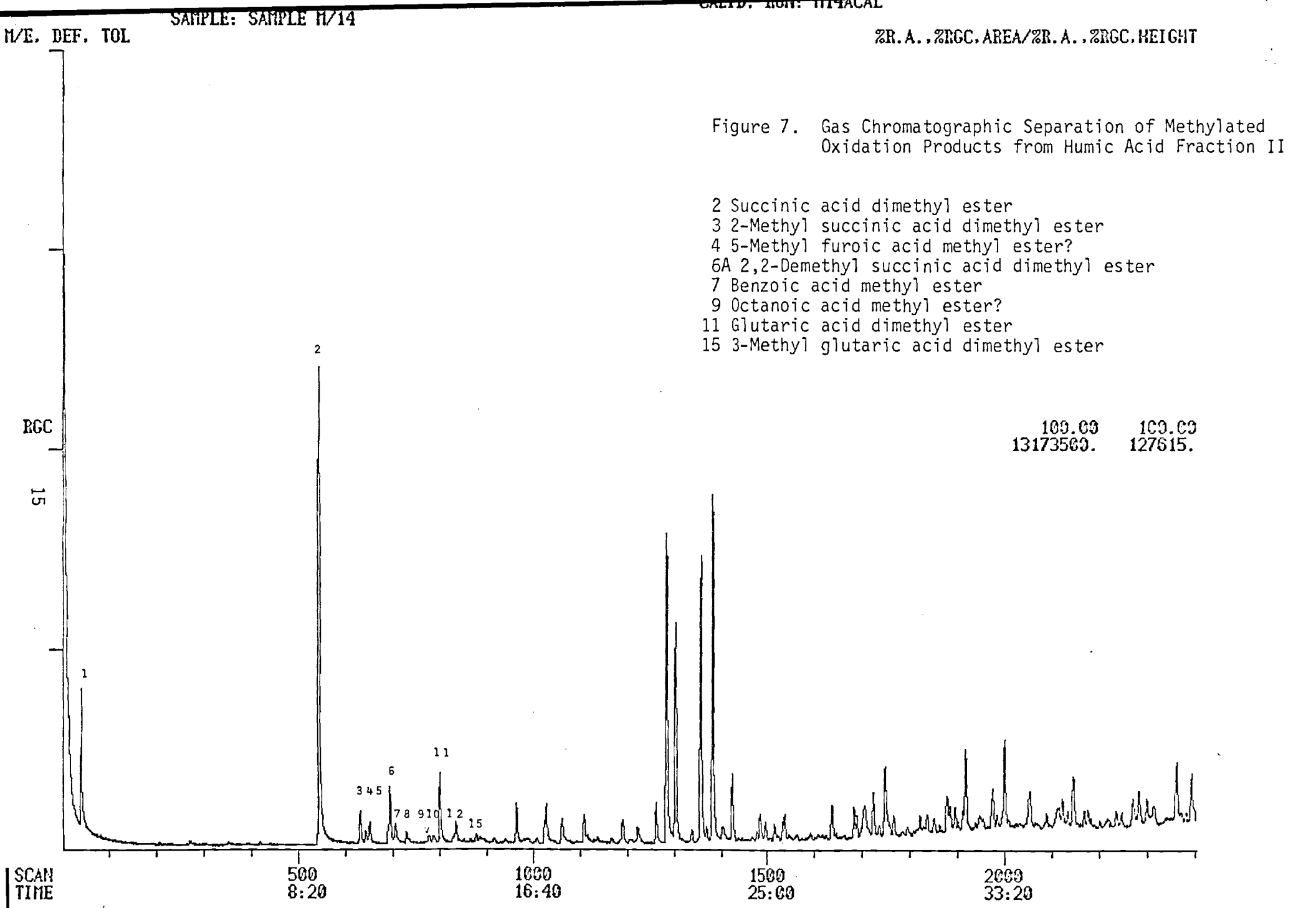
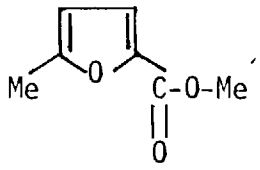


Figure 7. Gas Chromatographic Separation of Methylated Oxidation Products from Humic Acid Fraction II

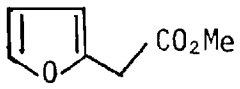
and 5-methyl furoic acid methyl ester, a satisfactory matches with reference spectra were not achieved and the assignments were made on the basis of our own interpretation of the data.

For example, the fragmentation pattern shown in Figure 10 which is the average of three scans taken across the top of a small gas chromatograph peak can be explained on the basis of either of the two structures and fragmentation schemes shown below:



5-Methyl furoic acid methyl ester

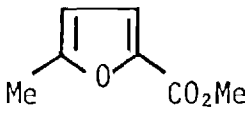
ml		
140	--	
125	M-15	Me
109	M-31	OMe
95	M-45	Cannot explain
81	M-59	Co_2Me
66	M-74	Co_2Me, Me



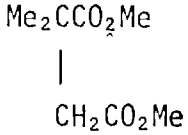
2-Furyl acetic acid methyl ester

140	--	
125	M-15	Me
109	M-31	OMe
95	M-45	Cannot Explain
81	M-59	Co_2Me
66	M-74	CH_3CO_2Me (via McLafferty rearrangement)

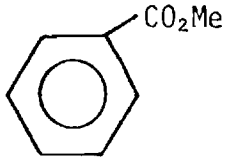
We are willing to consider other structures—especially if they explain the M/e 95 peak, but are otherwise resigned to comparison with authentic materials. Work on this and other problems of intepretation is continuing.



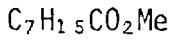
4) 5-Methyl Furoic Acid methyl ester?



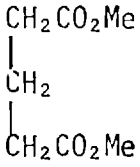
6a) 2,2-Dimethyl succinic acid dimethyl ester



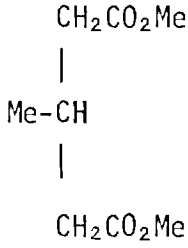
7) Benzoic acid methyl ester



9) Octanoic acid methyl ester?



11) Glutaric acid dimethyl ester



15) 3-Methyl glutaric acid dimethyl ester

Since some of these results represent new information regarding the structure of aquatic humic acids, a comparison with some of the literature is in order. Ogner and Gjessing[1] oxidized aquatic humics from Norwegian waters and reported the identification of only methyl esters of benzene carboxylic acids and their methoxy derivatives. Schnitzer and coworkers[24] have described the oxidation of soil humics and fulvics to get straight-chain C-12 to C-18 fatty acid methyl esters, straight chain dibasic acid esters with four to eight carbon atoms, and a number of aromatic producrs. Since these workers report the resolution of only 30-40 peaks using packed columns, the adVantages of our capillary techinques which have successfully resolved 120 peaks from similar reaction mixtures are quite obvious.

Six of the eight compounds described in this report have not been identified by this groud in any of their extensive oxidation work. The identification of a furan derivitive (4) which might derive its biogenetic origin from carbohydrates is interesting. In our case, the carboxylate function in the 2-position seems more reasonable than the reported[2] aldehyde function in the same position which would not be expected to survive he conditions of the original oxidation.

We intend to solidify these identifications by comparisons with authentic matierals whenever possible. An example of how the identification of these compounds is carried out is presented in Figures 8 and 9. Figure 8 shows an expansion of the total ion chromatogram for scans 500-599. This scale expansion capability makes it easier for the operator to select a representative scan (in this case scan No. 543) background subtraction and comparison with the computer's library of mass spectra. Figure 9 shows a match between the unknown spectrum and the best-fitting reference spectrum.

Matches were obtained in a similar fashion for six of the eight tentatively identified spectra. In the case of octanoic acid methyl ester

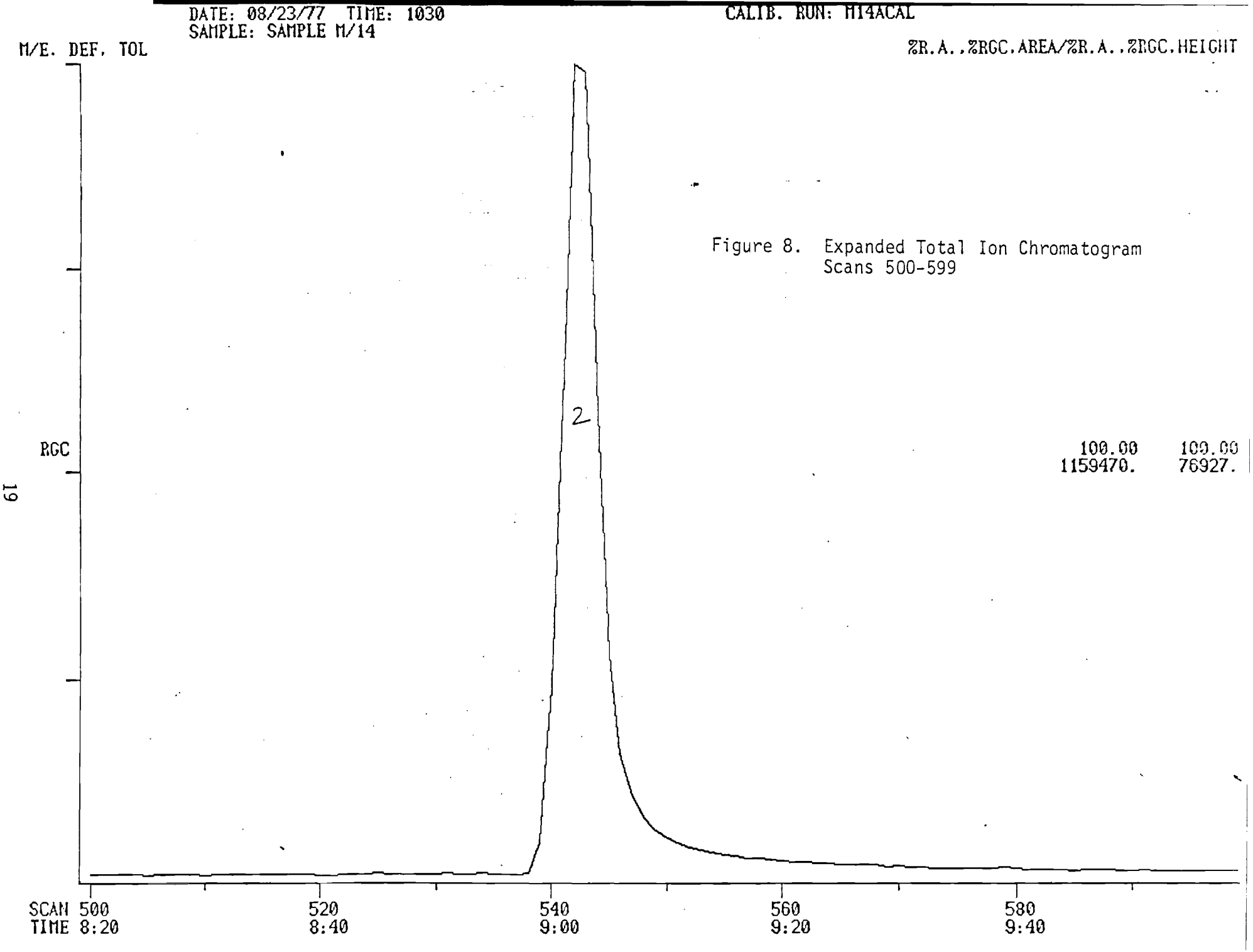


Figure 8. Expanded Total Ion Chromatogram
Scans 500-599

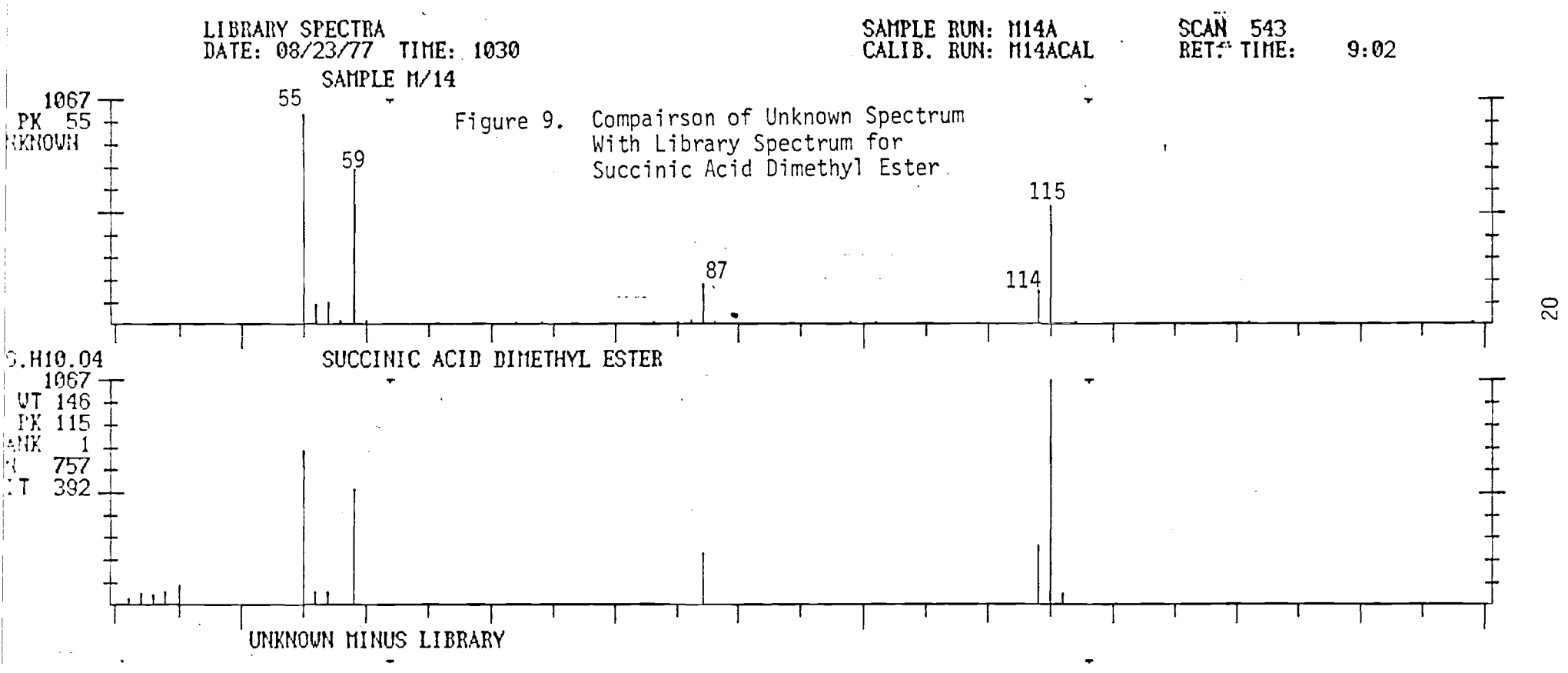


Figure 9. Compairson of Unknown Spectrum With Library Spectrum for Succinic Acid Dimethyl Ester

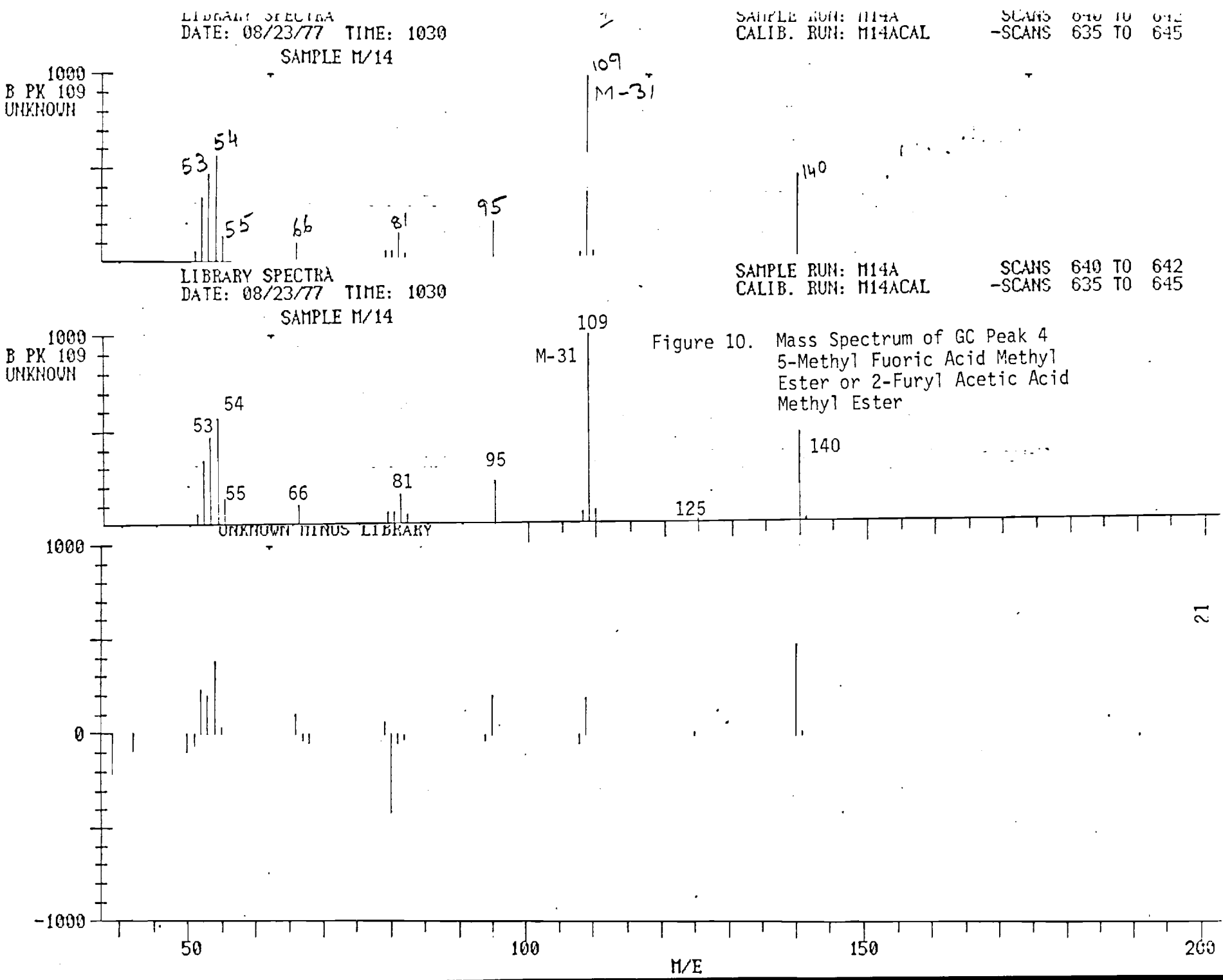


Figure 10. Mass Spectrum of GC Peak 4
5-Methyl Fuoric Acid Methyl
Ester or 2-Furyl Acetic Acid
Methyl Ester

V. MODEL COMPOUND STUDIES - SERIES I FACTORIAL EXPERIMENT

The series of reactions of coniferyl alcohol with chlorine under various pH, temperature and contact time conditions are proceding on a regular schedule. These experiments are carried out using the factorial approach outlined in the attachment titled "Series I - Factorial Experiment: sent to the sponsor on August 17 with the mid-monthly financial report.

The series of six samples which were described on pages 24-25 of the September monthly report are being used for procedural and analytical methodology development. Two types of control samples are being run in the factorial experiment series: a) a liter of high purity water containing coniferyl alcohol only, and b) a liter of high purity water containing buffer components and chlorine solution with no coniferyl alcohol.

The following sets of samples in the factorial samples have been prepared and taken to the final analysis state:

Set 1: pH 6.0; Temperature 25^{o}; Contact (methods development) Time 1 hour;
4-2.1 ± 0.1 mg coniferyl alcohol
5-5.0 ± 0.1 mg coniferyl alcohol
2- Controls

Set 2: pH 6.0; Temperature 10^{o}; Contact Time 1 hour;
2- 2.0 ± 0.1 mg coniferyl alcohol
2- Controls

Set 3: pH 7.5; Temperature 10^{o}C; Contact Time 1 hour;
2- 2.0 ± 0.1 mg coniferyl alcohol
2- Controls

The molar ratio of chlorine to coniferyl alcohol used in Sets 1-3 was 10:1. All samples in Sets 1-3 were quenched after the one hour contact time by adding a slight excess (based on amount of chlorine) of pentane-washed sodium sulfite.

The pH of the reaction mixtures was adjusted by adding 10 g of pentane-washed sodium carbonate.

The pentane extracts of the resulting pH 10 solution containing the basic and neutral components of the reaction mixtures were dried with pentane-washed anhyrdous sodium sulfate and concentrated to a volume of 1-2 ml.

The pentane-extracted reaction mixtures were acidified to pH 1 by the cautious addition of 10 ml of 16 M sulfuric acid and subsequently extracted with ether-benzene (1:1). The extracts were dried with ether-washed anhydrous sodium sulfate and treated with etheral diazoethane.

VI. REACTION OF POLYELECTROLYTES WITH CHLORINE

An exploratory study of the products which might be formed by the reaction of polyelectrolytes with chlorine was initiated. Samples of currently used anionic, cationic and non-ionic polyelectrolytes were obtained from the Nalco Chemical Company and the Dow Chemical Company. The polyelectrolytes were dissolved or suspended in 1 liter of high purity water at pH 6.5 and treated with 10 ml of a solution of 5 mg/ml of chlorine. A control sample containing polyelectrolyte and buffer without chlorine solution was prepared for each of the polyelectrolytes examined. The weights of the polyelectrolytes used were as follows:

Nalco # 7171	23.5 mg + 5 mg chlorine
	23.0 mg + 0 mg chlorine
Nalco # 8101	44.6 mg + 5 mg chlorine
	40.0 mg + 0 mg chlorine
Nalco # 7763	28.3 mg + 5 mg chlorine
	27.2 mg + 0 mg chlorine

The polyelectrolyte-chlorination reaction mixtures were not quenched with sodium sulfite. The resulting turbid suspensions were filtered through precleaned glass wool. The filtrates from the six samples were passed through a bed of Rohm and Haas XAD-2-macroreticular resin in order to isolate organic compounds. The adsorbed compounds were subsequently eluted

with ethyl ether. The resin was then purified by Soxhlet extraction with methanol and with benzene. The extracted resin was stored under methanol. The resin bed (20 g) was prepared in methanol and washed with high purity water before the polyelectrolyte-chlorine reaction mixture was added. After each ether elution (one 50 ml and two 25 ml portions), the resin bed was washed with 200 ml of high purity water. The ether eluates were dried over ether washed anhydrous sodium sulfate. The six samples will be analyzed by GC/MS and by LC in an effort to determine the compositon of polyelectrolyte-chlorine reaction products. Particular emphasis will be placed on the search for chlorinated compounds.

VII. OZONE EXPERIMENTS

A limited amount of effort continues to be devoted to experiments designed to better define the oxidant produced by various "ozone" generators. A series of comparisons of the products of 1) ultraviolet photon energy by "Photozone" and 2) corona discharge ozone were made using sulfuric acid and phosphoric acid as solvents. The ozone generator patented by Carlson (U.S. 4,035,657) produces pure ozone and no carbon dioxide if the plastic casement is replaced by an all-glass system. The ozone absorption maximum of the photozone discharge in sulfuric acid disappears and an increased transmissivity at short wavelengths is noted. The latter phenomenon may be due to the elimination of some weakly absorbing component in the sample cell by the action of the ozone or by some kind of unknown photoemission process. Since this phenomenon is observed only in the sulfuric acid, it would seem that this molecule must be implicated in the production of the observed phenomenon.

Examinations of the infrared spectra of the photozone discharge versus a corona discharge ozone was made using a Wilks long light path "Miran"

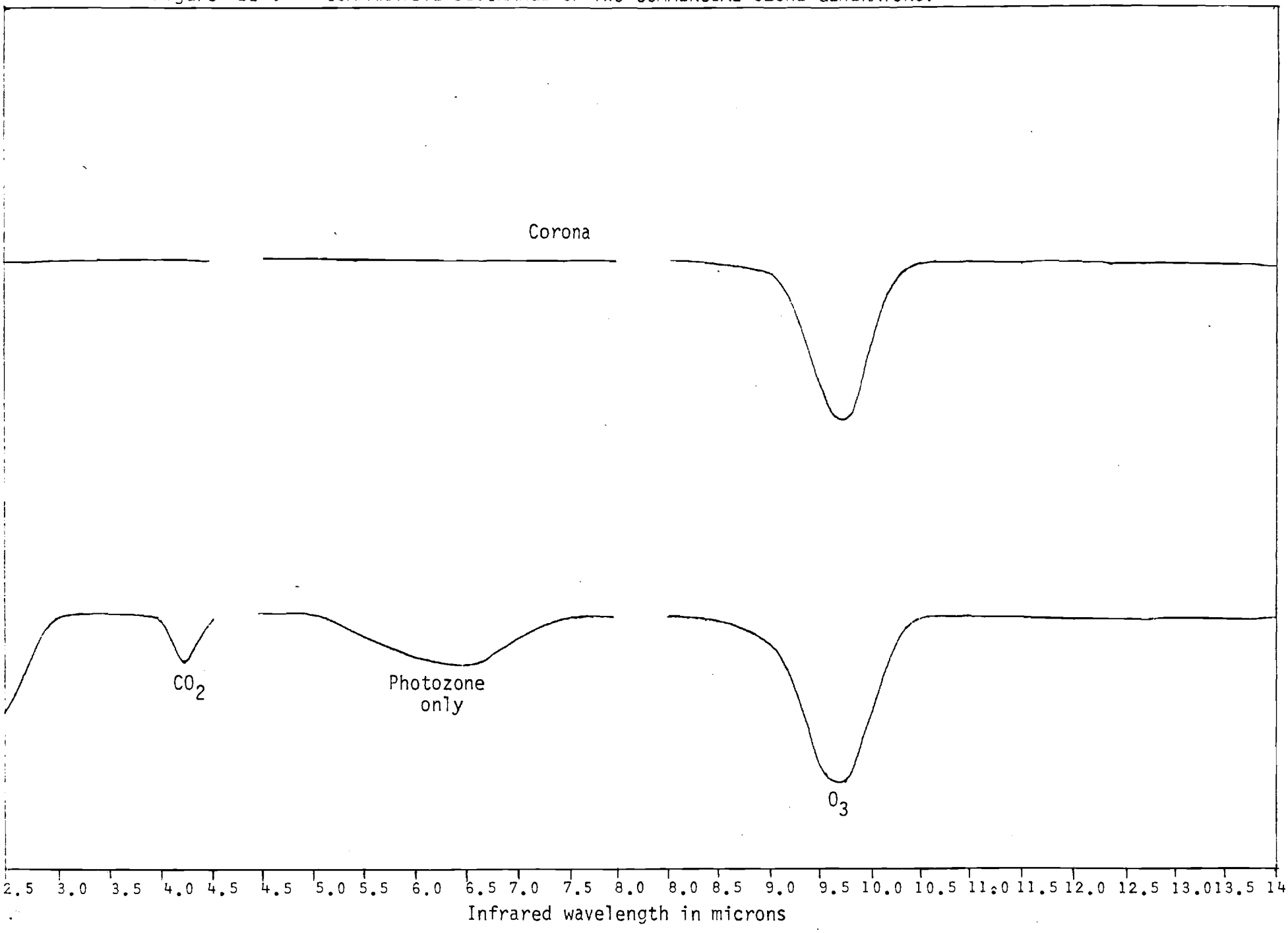
spectrophotometer. Absorption at 9.8μ (ozone) was observed in the gases from each generator. In addition, absorption at 4.3 μ was observed in the photozone discharge and is attributed to carbon dioxide from degradation of the plastic shield. An as yet unidentified absorption at 6.5 μ was also observed only in the photozone discharge. These results are shown in Figure 11

Future work of a similar fundamental nature regarding the identity of active oxygen species such as might be used for the treatment of drinking water will include: 1) some attempts to trap singlet oxygen with 2.5 dimethyl furan 2) the addition of singlet oxygen quenchers such as 1,4-diazabicyclo(2,2,2) octane and 3) possibly the addition of sensitizers to solutions of model compounds treated with the various types of ozone. This work is suggested by a recent publication by R. G. Zett, et al., in Nature Vol. 267, 421 (1977).

Background research for a paper to be presented by Dr. Ingols at the International Ozone Institute at Toronto in November uncovered the interesting possibility of active nitrogen species becoming involved in the treatment of water and wastewaters with ozone. For example, passing discharges through nitrogen is known to produce a persistent golden-yellow afterglow which is chemically very reactive. Evidence indicates that the chemical reactivity can be attributed to atomic nitrogen while the observed afterglow is produced by an excited nitrogen molecule which emits the radiation as it slowly reverts to the ground state.

Since most commercial ozone systems subject at least some nitrogen to the activation process and since active nitrogen might have a considerable potential for the generation of hazardous substances from trace organic materials, it would seem to be highly desirable to do a little preliminary work with active nitrogen.

Figure 11 . COMPARATIVE DISCHARGE OF TWO COMMERCIAL OZONE GENERATORS.



VIII. MISCELLANEOUS

Strategies mentioned in the last monthly progress report for the improved gas chromatographic resolution of ethylated syringic acid were not successful. A glass column is being fabricated so that further work may proceed if current problems are being caused by the use of metal columns. The gas chromatographic analysis of ethylated and silylated derivatives of coniferyl alcohol and hesperitin can also be expected to be improved by the use of all-glass systems.

References

1. Ogner and Gjessing, Beoderna, 14, (1975), 139.
2. Schnitzer, and Vendette, Can. J. Soil Sci., 55 (1975), 93.
3. Ortiz de Serra and Schnitzer, Soil Biol. Biochem., 5 (1973), 287
4. Matsuda and Schnitzer, Soil Sci., 185 (1972).

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report
November 4, 1977

by

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DISCLAIMER

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I. PERSONNEL

No personnel changes have taken place during this reporting period.

II. EQUIPMENT

We are continuing to advance our familiarity with the capabilities of the new Finnigan 4023 GC/MS system. Start-up problems seem to have settled down to sensible levels. We are now taking advantage of some of the more sophisticated software packages to help clean up the data already on file and thus make it more amenable to interpretation. For example, a comparison of the earlier total ion chromatograms for the oxidation experiment (shown in Figures 1 and 2) with the computer-enhanced chromatograms (shown in Figures 3 and 4) shows a considerable improvement in baseline and sharpness of peaks.

Those few pieces of equipment needed for the mini-pilot facility are now being fabricated by our glassblowing shop. Although it may not have been obvious from last month's report, it should be emphasized that this facility is being set up for maximum flexibility so that changes in the treatment sequence can be readily made. Furthermore, it will be possible to sample before and after each step in the sequence.

Current plans for use of the facility call for an initial shakedown with ppm levels of either hesperitin or coniferyl alcohol followed by a series of investigations with model compounds in which samplings for analysis of chorinated organics and trihalomethanes will be conducted at various points in the treatment sequence. Appropriate tests will be conducted at the start of each series of experiments in order to determine the proper levels of

Figure 4. Total ion chromatogram, methylated oxidation products from humic acid fraction II.
Scans 1-1800 (not enhanced)

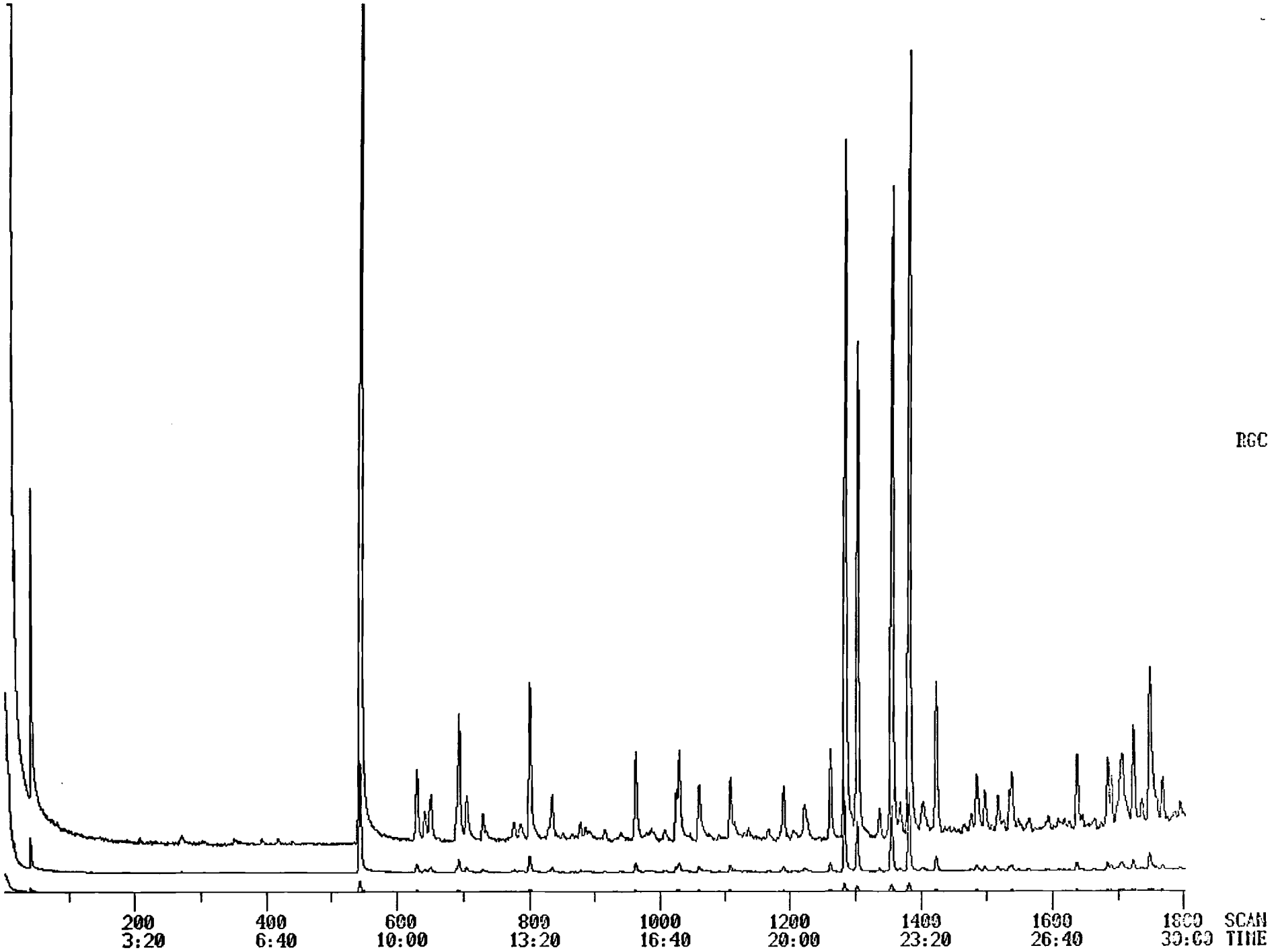


Figure 2. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II.
Scans 1-1800 (enhanced).
NOTE: Disappearance of Tailing.

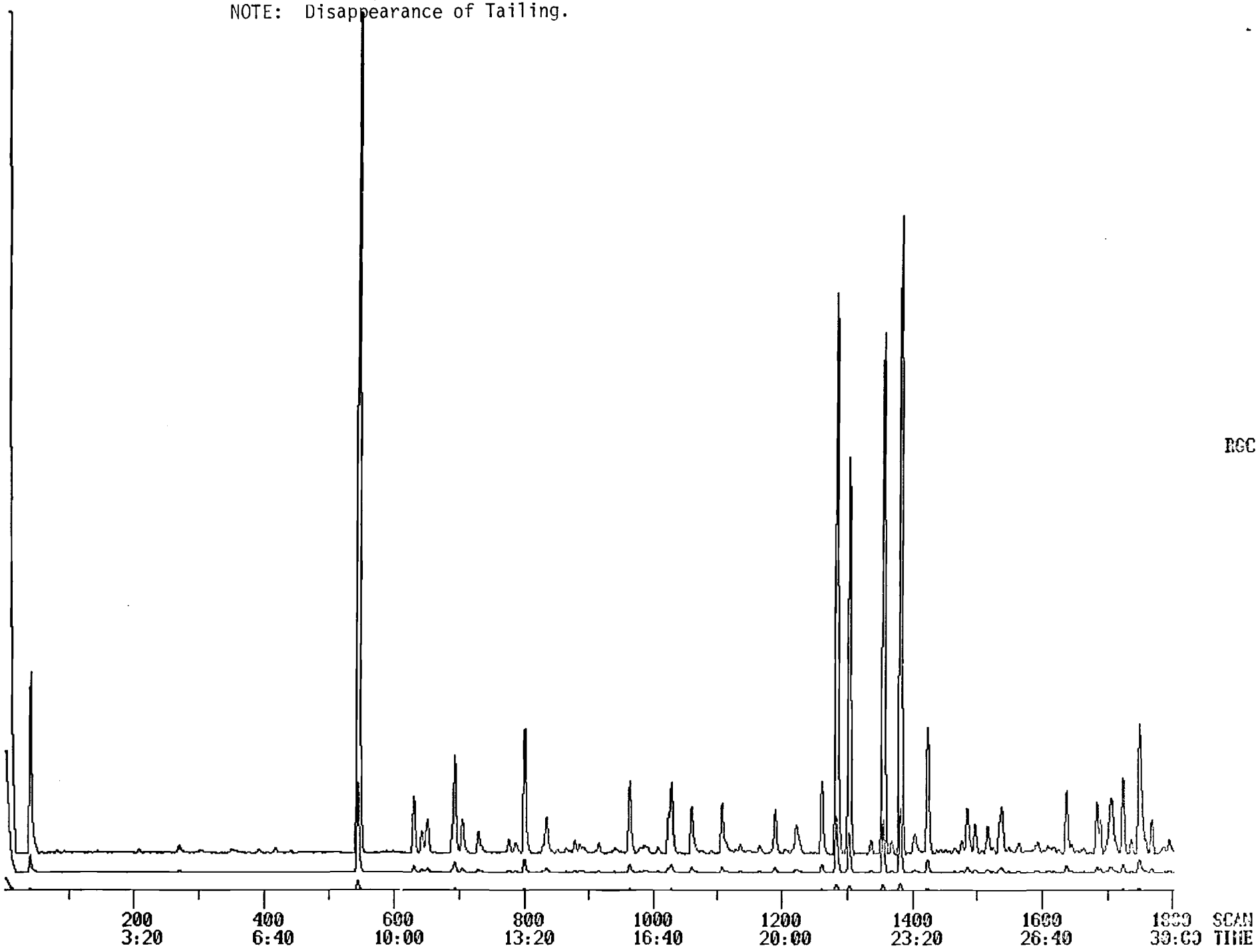


Figure 3. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II. Scans 1800-3507 (not enhanced).

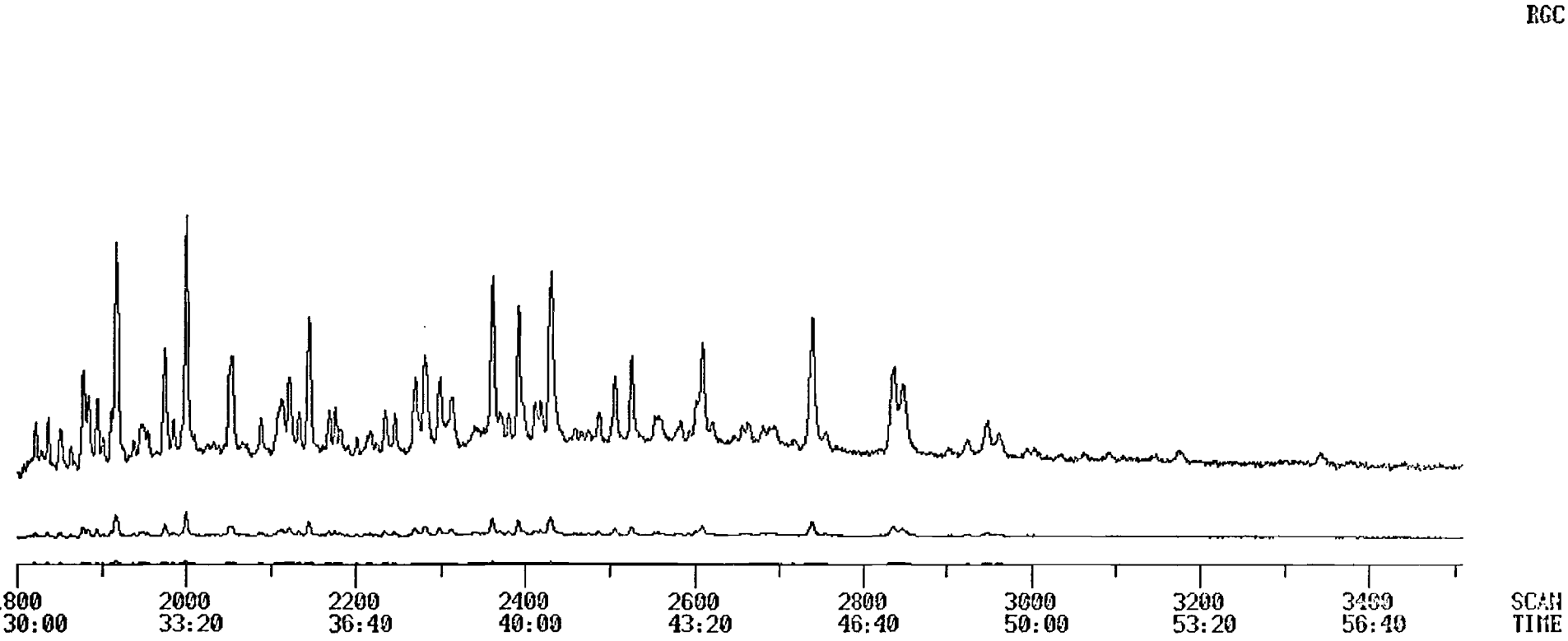
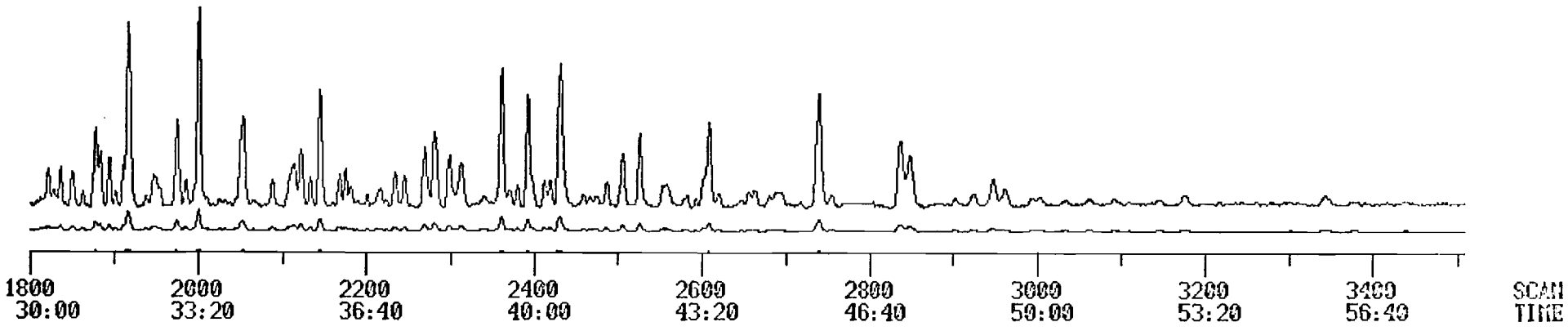


Figure 4. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II. Scans 1800-3507 (enhanced).
NOTE: Improved Baseline.



flocculants, chlorine, etc. which should be added. Both system and reagent blanks will be run in an effort to guard against contamination. If evidence of contamination is found in these early tests, the source will be systematically isolated and eliminated by reagent or subsystem as the case may be.

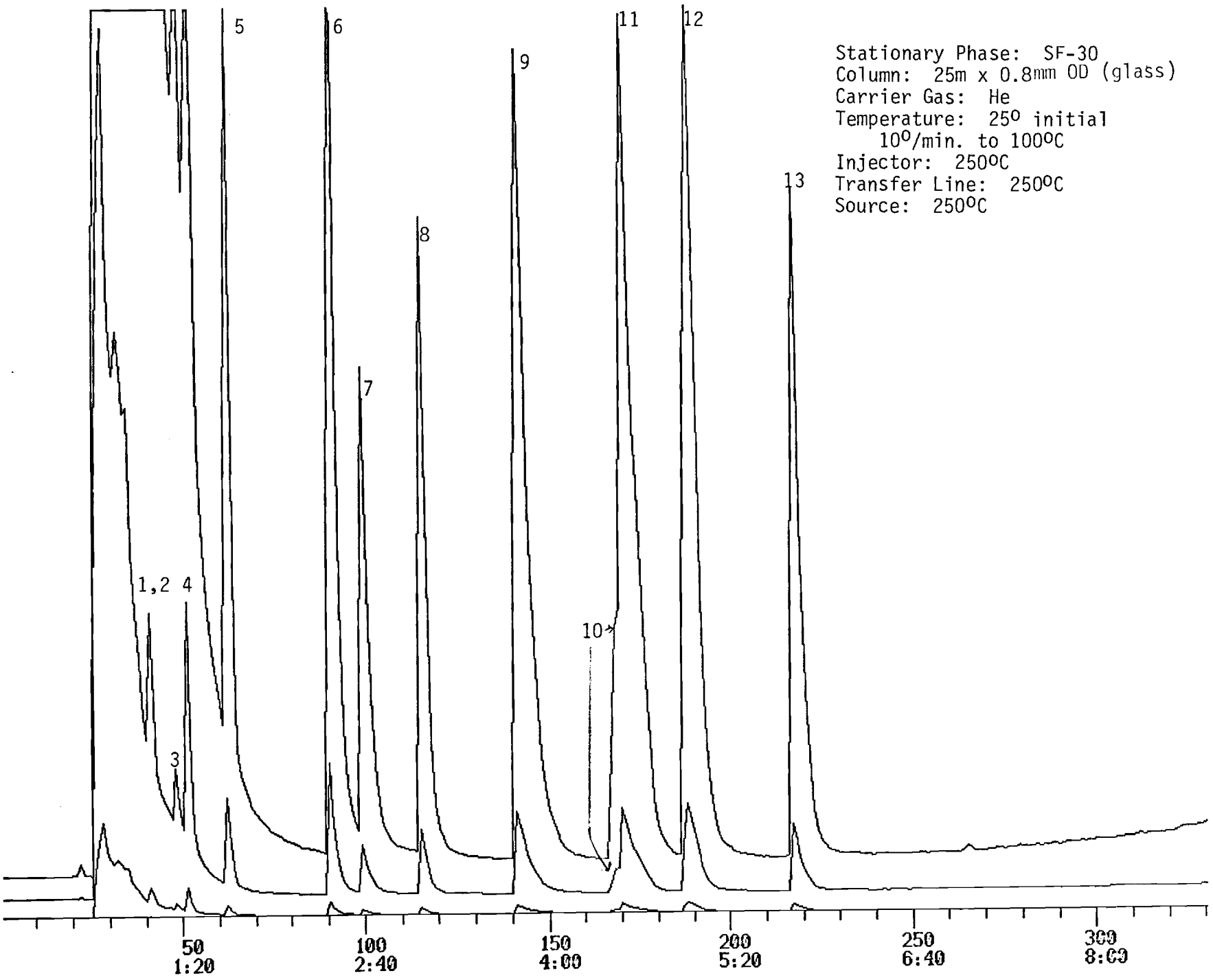
Once the preliminary systems and reagent-testing experiments have been completed, the effects of changes in the reaction conditions such as chlorine dosage, concentration of model compounds, pH, type and concentration of flocculants, contact times and sequence of steps will be investigated. This system offers a great deal of flexibility so that suggestions from the sponsor can be readily incorporated into the program (i.e., slow sand filtration).

III. GAS CHROMATOGRAPHIC STUDIES

The advantages of capillary column techniques have become increasingly evident as this research begins to move out of the preliminary stages. We are in the process of outfitting our new Finnigan gas chromatograph (not to be confused with the one attached to the mass spectrometer) with a capillary injector and detector interface so that we will not have to continue to tie up the mass spectrometer for capillary column methods development. We are presently using a 25 meter x 0.8 mm (OD) glass capillary column coated with SE-30. A test run was carried out with a mixture of several trihalomethanes, chlorinated organics and other volatile organic compounds in pentane—each at a level of 1-4 micrograms. Figure 5 shows the total ion chromatogram for the separation of the following components. Library confirmation or comparison with authentic, separately prepared standards was achieved in every case.

1. Bromochloromethane } not resolved
2. Chloroform
3. 1, 2 Dichloroethane
4. Benzene
5. Dichlorobromomethane
6. Toluene
7. Dibromochloromethane
8. Tetrachloroethylene
9. Chlorobenzene
10. Bromoform (leading shoulder)
11. Xylene Isomer
12. Xylene Isomer
13. Cumene

We are presently engaged in working out conditions for lowering the detection limits for the trihalomethanes via the techniques of selected ion monitoring so that we will be able to do a better job of monitoring these compounds at the levels expected to be produced in our factorial and mini-


Stationary Phase: SF-30
Column: 25m x 0.8mm OD (glass)
Carrier Gas: He
Temperature: 25° initial
10°/min. to 100°C
Injector: 250°C
Transfer Line: 250°C
Source: 250°C
1,2
3
4
5
6
7
8
9
10
11
12
13
RGC
50
1:20
100
2:40
150
4:00
200
5:20
250
6:40
300
8:00
SCAN
TIME

pilot studies. Additional columns will be purchased in order to increase our ability to handle a wider range of naturally occurring organic compounds.

IV. COLLECTION OF RIVER WATER SAMPLES

The sampling site is located 1.3 miles (2.7 km) east of the Satilla River (N 31^{o} 05' W 81^{o} 52'), where route 259 crosses the county line between Camden and Brantley Counties. About 416 liters of water were collected in steel drums (55 gals. each) from a small stream draining a swamp occupying the Satilla River flood plain.

This particular site has been chosen for the following reasons: river water in general is made up of two component waters, a) surface runoff and b) subsurface or groundwater flow. According to Reuter and Perdue (1977) it is the surface runoff component that is responsible for contributing the bulk of the organic matter to river water, whereas groundwater typically is very low in organic carbon. Our sampling site delivers surface runoff water high in organic carbon content. Just 100 m to the north of our sampling site a borrow pit has recently been excavated. It is filled with groundwater of very low organic carbon content. Both of these waters are flowing into the nearby Satilla River. Obviously, sampling the organic-rich surface runoff component offers the advantage of obtaining samples with a considerable higher concentration of organic matter. Moreover, geochemically one is still dealing with a true river water component.

V. ISOLATION OF AQUATIC HUMIC MATTER

River water was passed through a glass wool filter to remove suspended solids and subsequently acidified with concentrated hydrochloric acid to pH 1.7 (in order to fully protonate the dissolved acidic humics) before

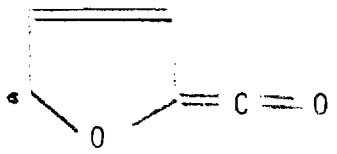
being passed over Amberlite XAD-7 resin (2.5 x 50 cm column). The original spectrophotometric absorbance of the water at 425 nm was 0.24 (a measure of organic content) which was reduced after passage over the XAD-7 to 0.02. The absorbance of the effluent was monitored as a check on the possible exhaustion of the absorbent resin. When the absorbance of the effluent rose, the XAD-7 column was washed with 100 ml of distilled water (to remove the acidic interstitial solution) and then eluted with an aqueous solution of purified triethylamine (33 ml/l). The excess triethylamine was removed under reduced pressure (rotary evaporator). This procedure resulted in a 70-fold increase in concentration of aquatic humics. This concentrate was then lyophylized. It yielded an amount of dry product equivalent to a concentration of 114 mg/l in the original water. However, this weight is now recognized as being partially inflated by the formation of triethylammonium—humate, as indicated by an increased N and H content. At this time we are treating the triethylammonium salt with a strongly acidic cation exchanger to replace $(C_2H_5)_3NH^+$ with H^+. The lyophilized sample was found to be essentially ash-free, an apparent result of the removal of associated metals during the acidification-adsorption process.

VI. CONTINUING IDENTIFICATION OF OXIDATIVE DEGRADATION PRODUCTS

It will be recalled that the processing of GC/MS data from the methylated oxidation products from humic acid fraction II has already resulted in the identification of the following products:

Peak Number	Product
2	Succinic acid dimethyl ester
3	2-Methyl succinic acid dimethyl ester
4	5-Methyl furoic acid methyl ester
6A	2, 2-Dimethyl succinic acid dimethyl ester
7	Benzoic acid methyl ester
9	Octanoic acid methyl ester?
11	Glutaric acid dimethyl ester
15	3-Methyl glutaric acid dimethyl ester

We now favor the 5-methylfuroate structure for peak 4 on the basis of the m/e 95 peak having the structure shown below. Since it is difficult to envision a structure of this type arising from the 2-furyl acetate, the alternate structure is now our first choice.



The following additional compounds have been identified by further processing of the same GC/MS data. The reader is referred to Figures 6, 7, 8 and 9 for the location of the identified peaks in the total ion chromatogram. It might also be pointed out that the increased definition of peaks by the computerized enhancement and scale-expansion techniques makes it quite evident that more than 119 separate peaks have been resolved. For the time being, the original numbering system will be retained.

Peak Number	Product
25	Dimethyl adipate
28	Dimethyl tartarate
35	Methyl 4-methoxybenzoate
41	Dimethylphthalate
43	Dimethylterephthalate
44	Dimethylisophthalate

The above identifications were made by matching with library spectra. In addition, the following structures were arrived at independently:

54	Methyl dimethoxybenzoate isomer
77	Trimethyl benzenetricarboxylate isomer
79	Trimethyl benzenetricarboxylate isomer
89	Undecane
97	Tetramethyl benzenetetracarboxylate isomer

It will be noted that the orientation of the substituents in peaks 54, 77, 79

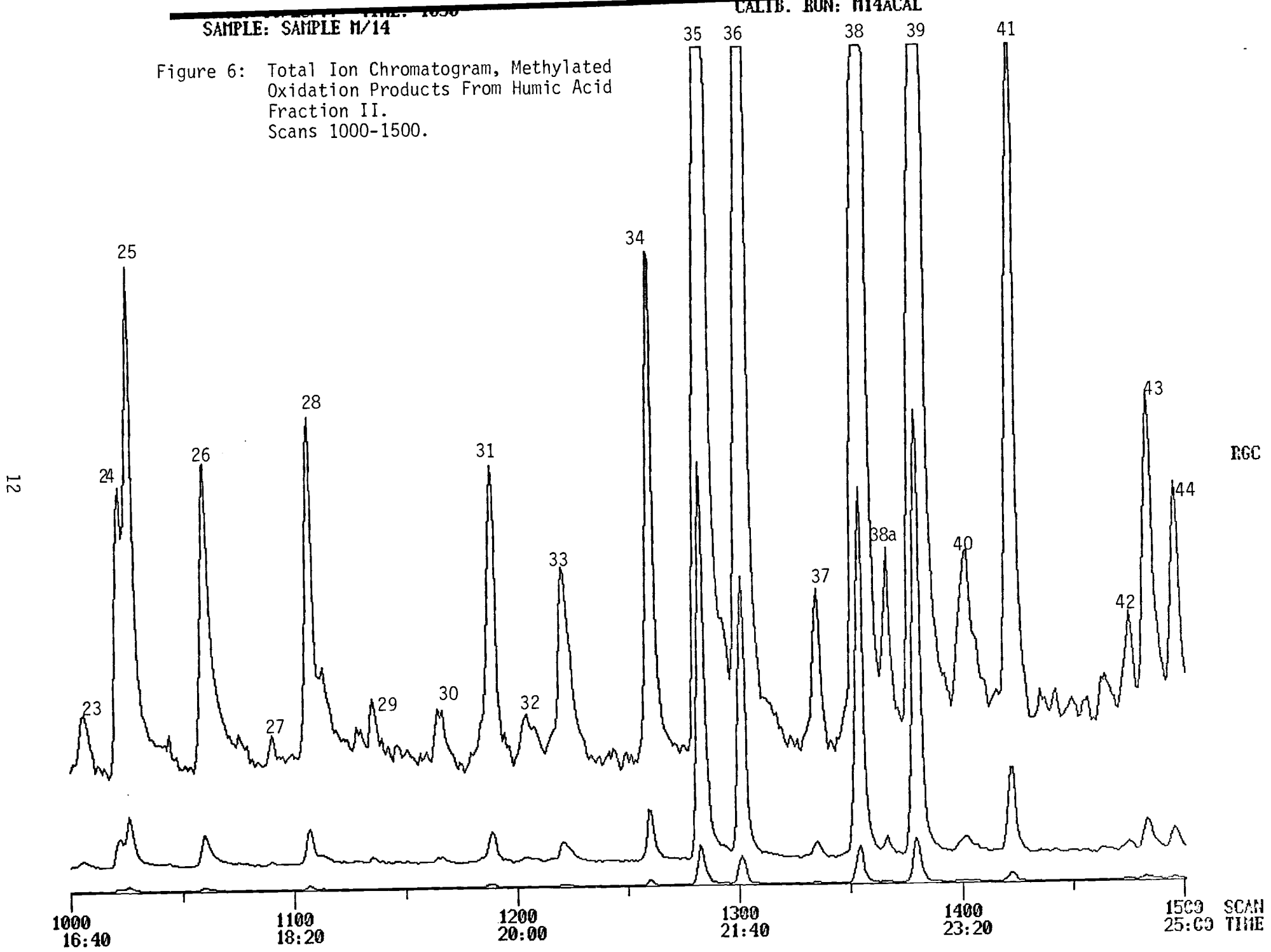


Figure 6: Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II.
Scans 1000-1500.

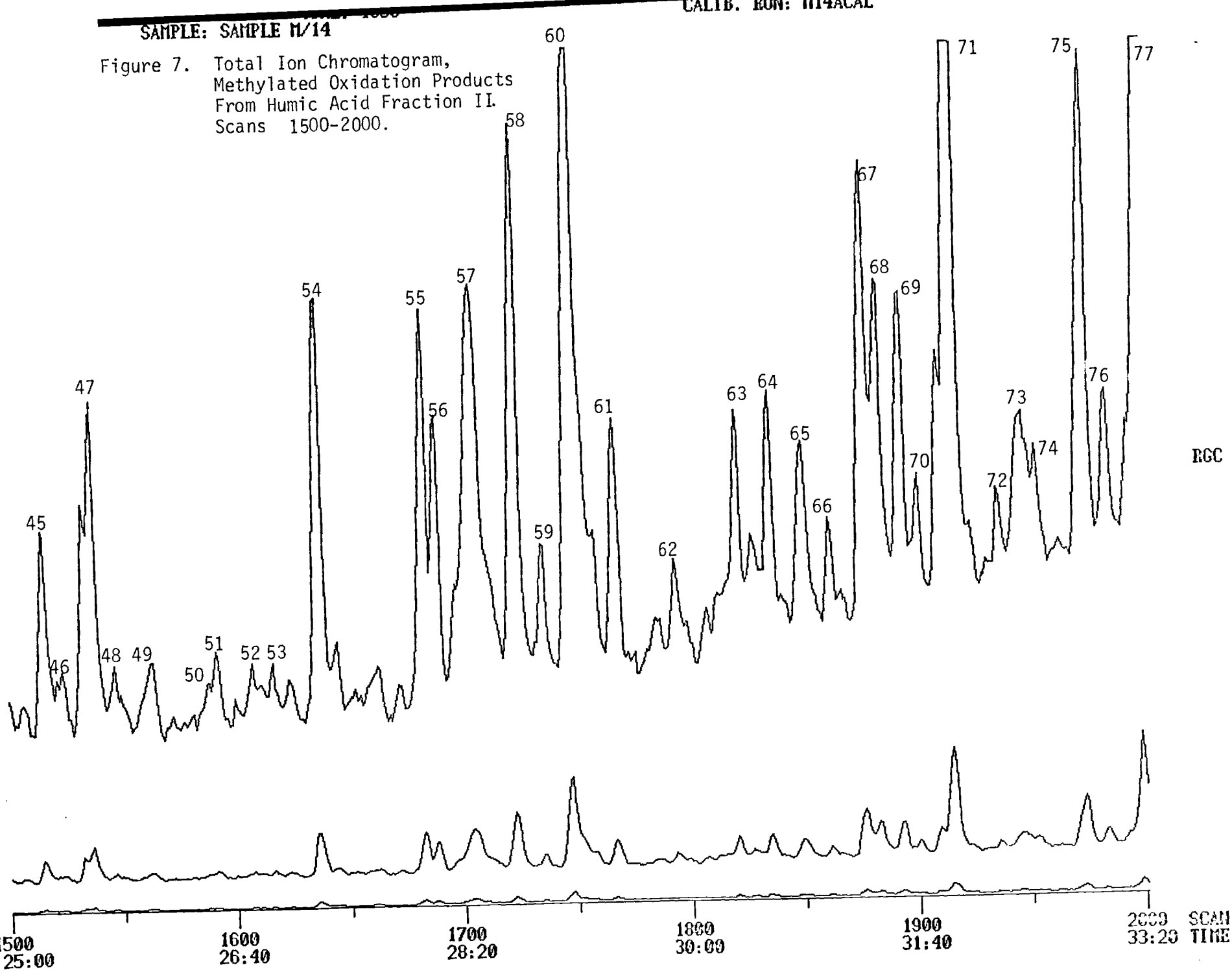
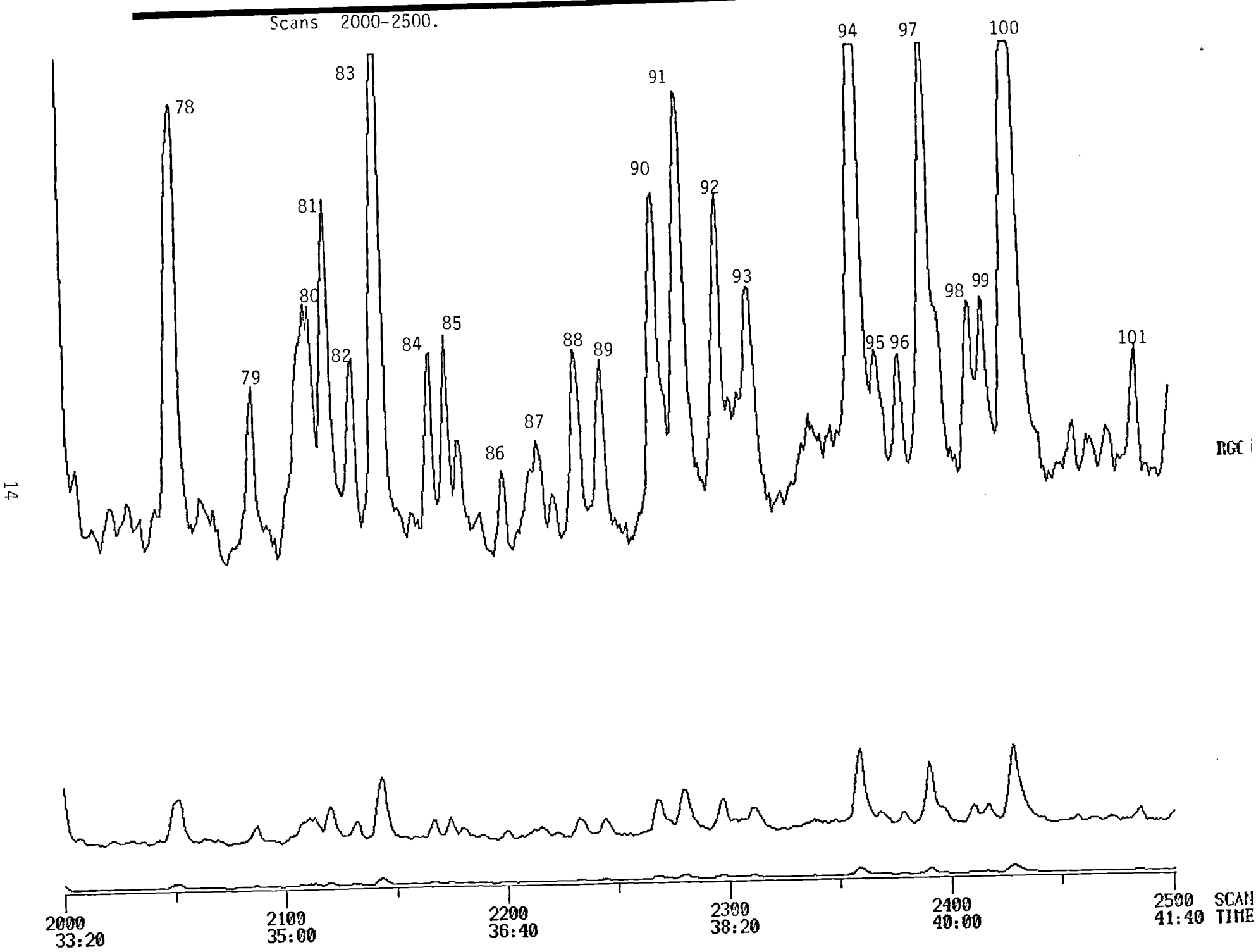


Figure 7. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II. Scans 1500-2000.


Scans 2000-2500.
78
79
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86
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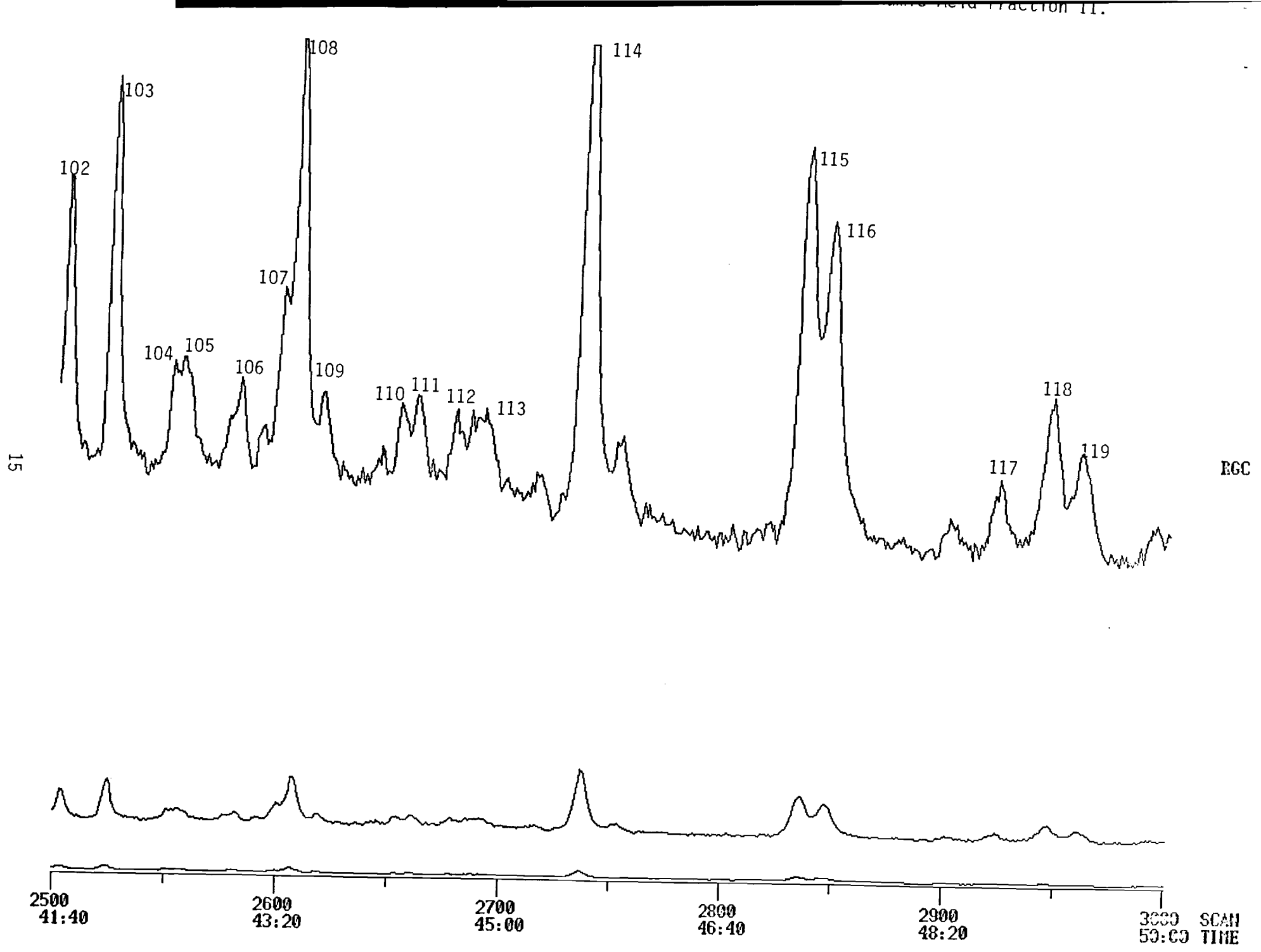
92
93
94
95
96
97
98
99
100
101
RGC
2000
33:20
2100
35:00
2200
36:40
2300
38:20
2400
40:00
2500
41:40
SCAN
TIME


102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
15
RGC
2500
41:40
2600
43:20
2700
45:00
2800
46:40
2900
48:20
SCAN
TIME

and 97 could not be assigned. It is of further interest to point out the presence of a long chain hydrocarbon, undecane, in the oxidation products mixture. Rather than being a structural unit in the humic molecule, this alkane probably occurs in the original humic materials and is set free only upon oxidation. There is some evidence of other long straight chain compounds eluting between peaks 63 and 71 in the total ion chromatogram.

Ogner and Schnitzer (1970) have previously reported the presence of trapped hydrocarbons in soil fulvic acids, extractable only after methylation, ie., degradation of hydrogen bonding within the humic molecule. It may be that the "tertiary" structure of the humic acid molecules creates cavities of just the right size (much like those created by urea in urea inclusion compounds) into which normal alkane molecules can fit. Once such an association has been established, it would be difficult to disrupt short of breaking up the hydrogen bonding which created the cavity in the first place. This phenomenon could explain why humic substances form such strong associations with alkanes and fatty acids featuring a straight chain of seven or more methylene groups. In this way humic matter can act as a vehicle for the transport of hydrophobic substances in the aquatic environment.

Key spectra are presented in Appendix A.

[1]Ogner, G. and Schnitzer, M. (1970). The occurrence of alkanes in fulvic acid, a soil humic fraction. Geochim. Cosmochim. Acta 34, 921-928.

VII. IMPROVED PREPARATION OF DIAZOETHANE

The method for preparing diazoethane in hexane described in the Environmental Protection Agency Pesticide Manual (Section 5A(4)(C), Revised 12/3/74) was modified to use pentane in place of hexane. The effect of this modification was checked by the reaction of excess benzoic acid with the resulting diazoethane dissolved in pentane.

A solution of 2.3 g of potassium hydroxide in 2.3 ml of high-purity deionized water was brought to room temperature. Magnetic stirring was initiated and 30 ml of distilled-in-glass pentane was added. The flask and contents were placed in a freezer at -18°C for 30 minutes. The cold flask was replaced on the magnetic stirrer, rotation of the stirring bar was begun, and 1.60 g of solid N-nitroso-N-ethyl-N'-nitroguanidine was added in portions over a period of five minutes. Stirring was continued for an additional two minutes, after which time the deep yellow pentane supernatant was decanted from the aqueous phase (containing some fluffy solid matter) into a precooled (-18°C) flask containing 1.49 g of benzoic acid in 25 ml of ethyl ether. The yellow color was discharged rapidly with effeverescence.

The excess benzoic acid was extracted from the pentane-ether solution with cold 0.5 N aqueous sodium bicarbonate solution. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated in a Kuderna-Danish apparatus. The resulting liquid which was not completely free of low boiling components weighed 2.54 g.

Acidification of the sodium bicarbonate solution with 3.2 N sulfuric acid gave a precipitate which was collected by filtration, washed with water and dried in air. The benzoic acid recovered weighed 0.389 g and melted at 119-121°C. An additional 0.080 g of solid was obtained by

extraction of the filtered, acidified sodium bicarbonate solution with ethyl ether, followed by drying and concentration.

The recovered benzoic acid weighed 0.469 g; the benzoic acid which reacted with the diazoethane represented 1.496-0.469 or 1.027 g. Therefore, the yield of diazoethane formed in this experiment was 0.083 moles or 83%.

IX. MODEL COMPOUND STUDIES-SERIES I FACTORIAL EXPERIMENT

We have adopted an air of increased caution in our conductance of these experiments partly because we are still involved in developing the analytical methods needed to generate the necessary quantitative results and partly because we have uncovered evidence of contamination somewhere in the reaction sequence.

The neutral extracts from the chlorine-treated coniferyl alcohol reaction mixtures were analyzed by capillary GC/MS using the SE-30 column described earlier in this report. While chloroform was not found in these preliminary experiments, the total ion chromatogram shown in Figure 10, which is typical of several taken during the course of this work, did show two major components at scan numbers 430 and 450. The larger of these has been identified as BHT (Butylated Hydroxy Toluene or 2,6-di-t-butyl-4-methylphenol) by comparison with library spectra. These data are presented in Figure 11. Since this compound is a commercial antioxidant, it must be present in one of the reagents or else it is being introduced as the reaction is worked up. In either case, this problem must be resolved before the work can continue. The smaller peak in the total ion chromatogram at scan number 430 appears to be a closely related compound, perhaps an isomer. The mass spectra corresponding to the minor peaks in the total ion chromatogram were not sufficiently intense to permit identification. The contaminants appear in the blank containing only the coniferyl alcohol and in the blank containing all other reagents as well as in all of the reaction mixtures. At this time the most likely sources for this compound would seem to be 1) the pentane itself or 2) the vials used to hold the samples.

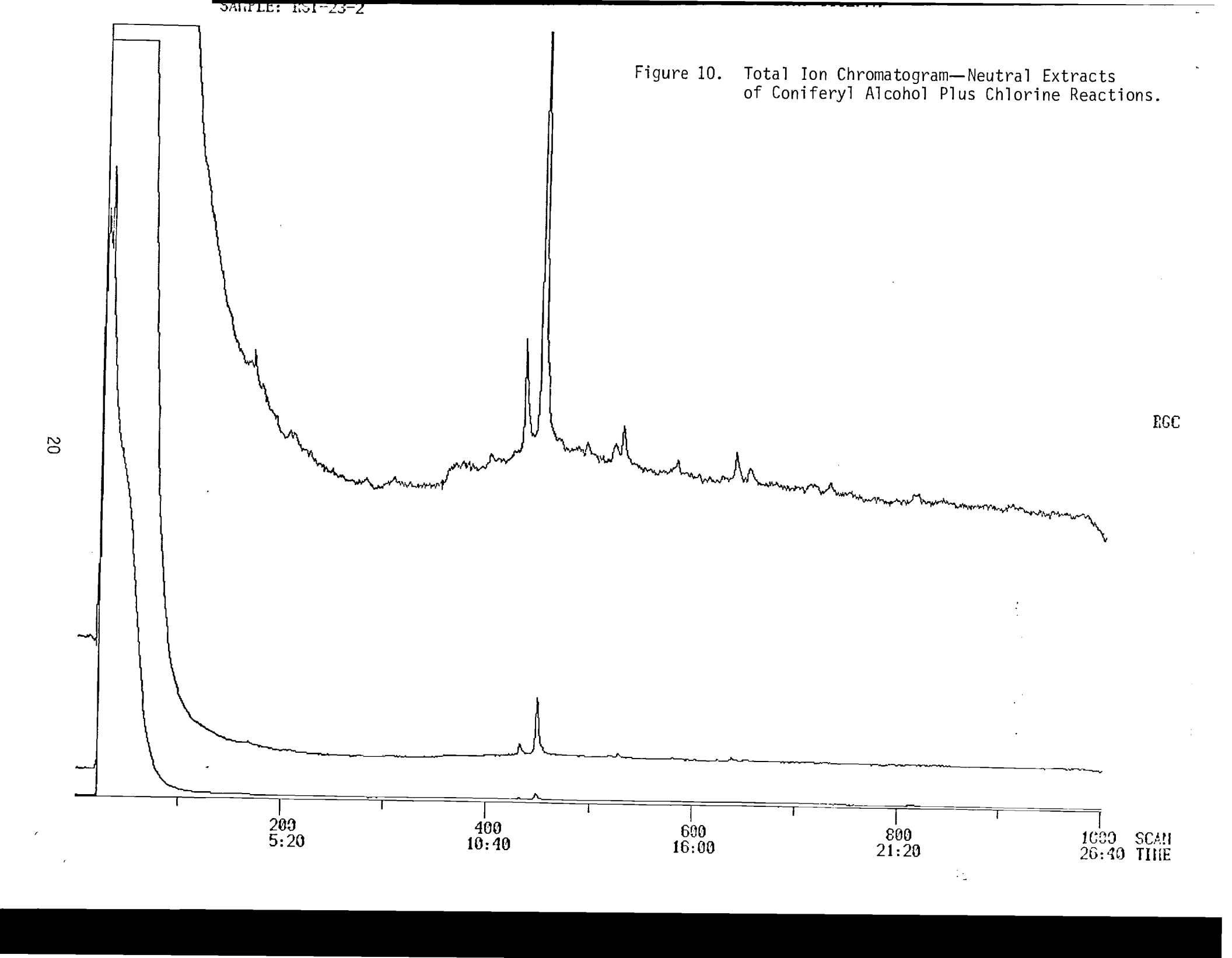


Figure 10. Total Ion Chromatogram—Neutral Extracts of Coniferyl Alcohol Plus Chlorine Reactions.

RSI-23-4

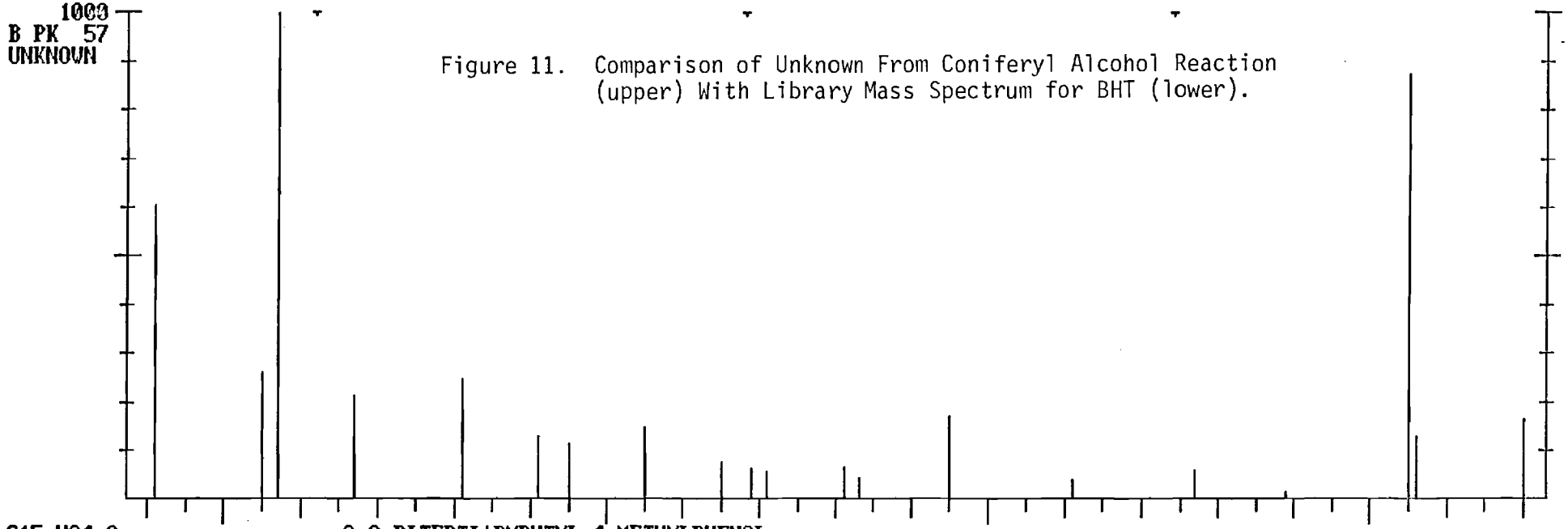
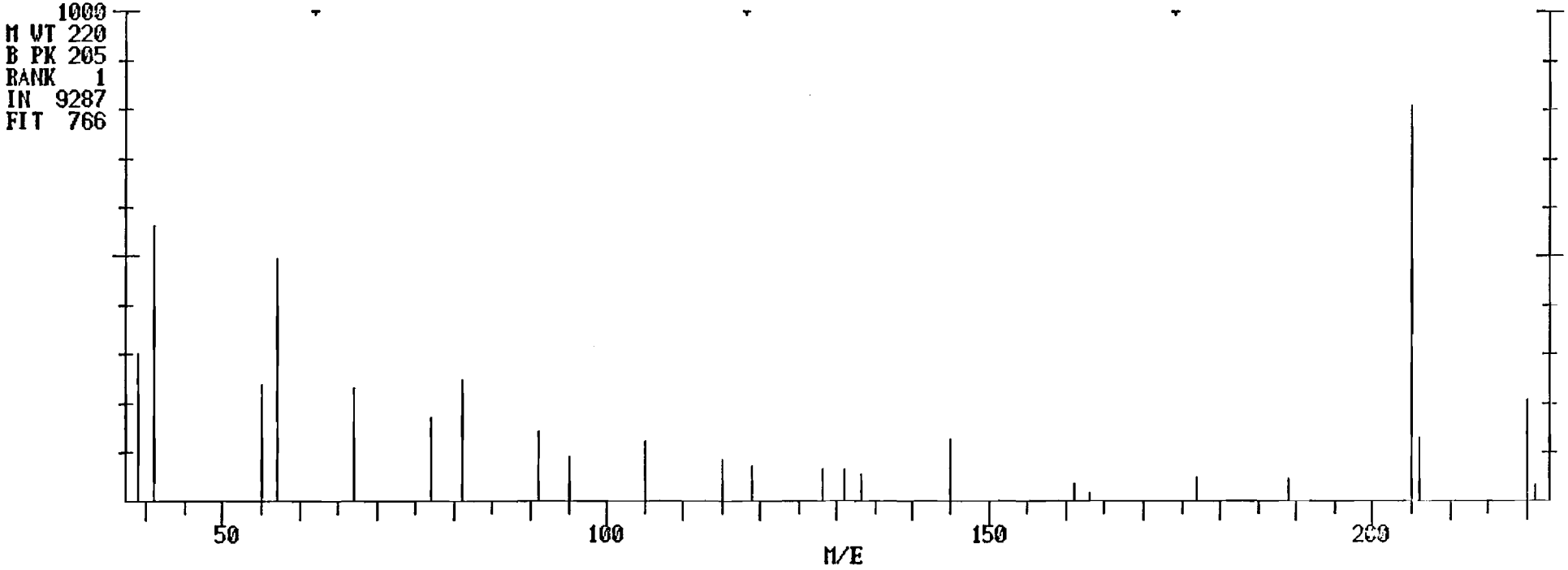


Figure 11. Comparison of Unknown From Coniferyl Alcohol Reaction (upper) With Library Mass Spectrum for BHT (lower).

C15.H24.O 2.6-DITERTIARYBUTYL-4-METHYLPHENOL



APPENDIX

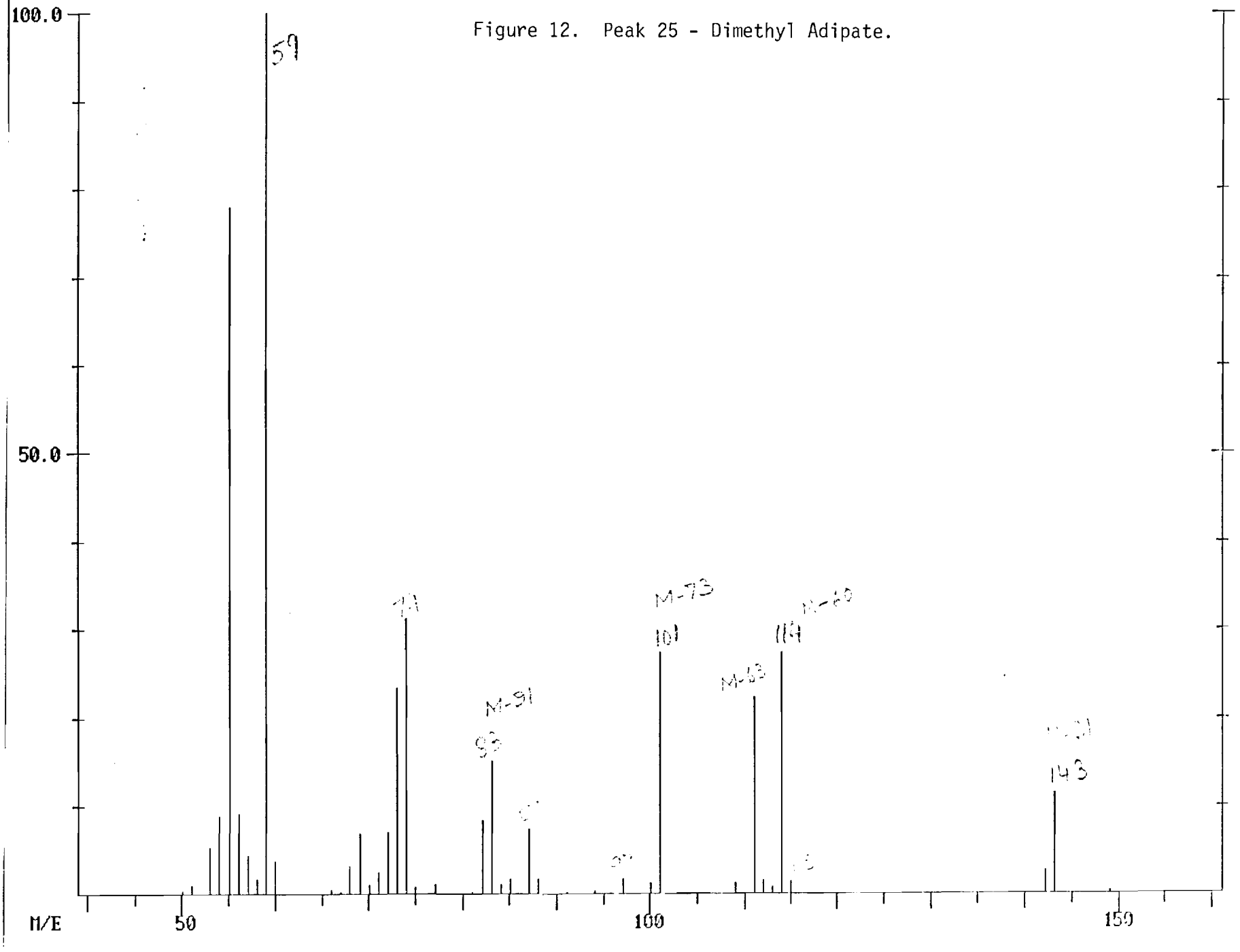
Key Mass Spectra

DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

CALIB. RUN: M14ACAL -SCANS 1022 TO 1037
BASE M/E: 59 TOTAL IONIZATION: 3885.

INT
938.

Figure 12. Peak 25 - Dimethyl Adipate.



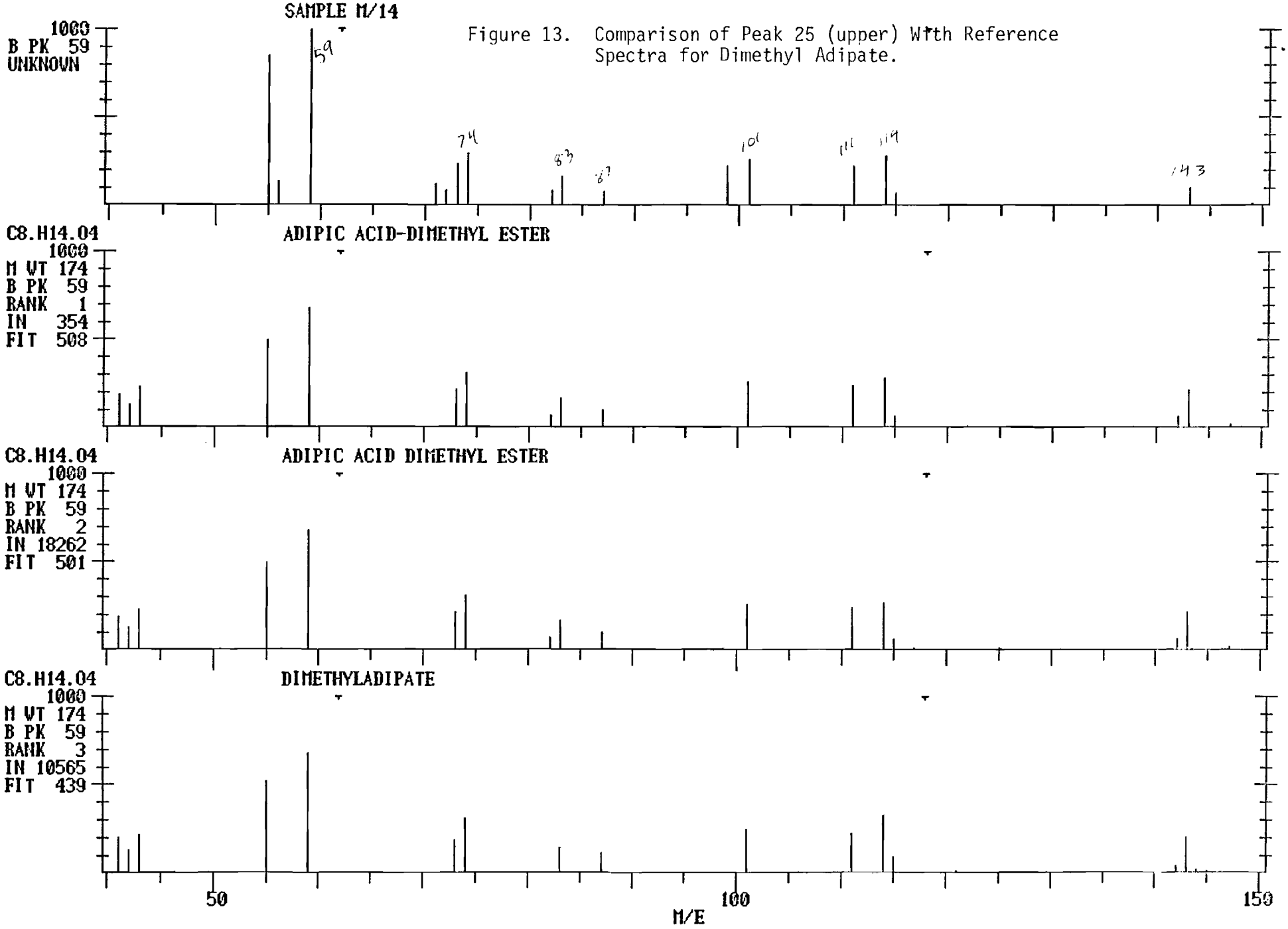


Figure 13. Comparison of Peak 25 (upper) With Reference Spectra for Dimethyl Adipate.

DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A SCANS 1106 TO 1107
CALIB. RUN: M14ACAL -SCANS 1109 TO 1111
BASE M/E: 103 TOTAL IONIZATION: 3750.

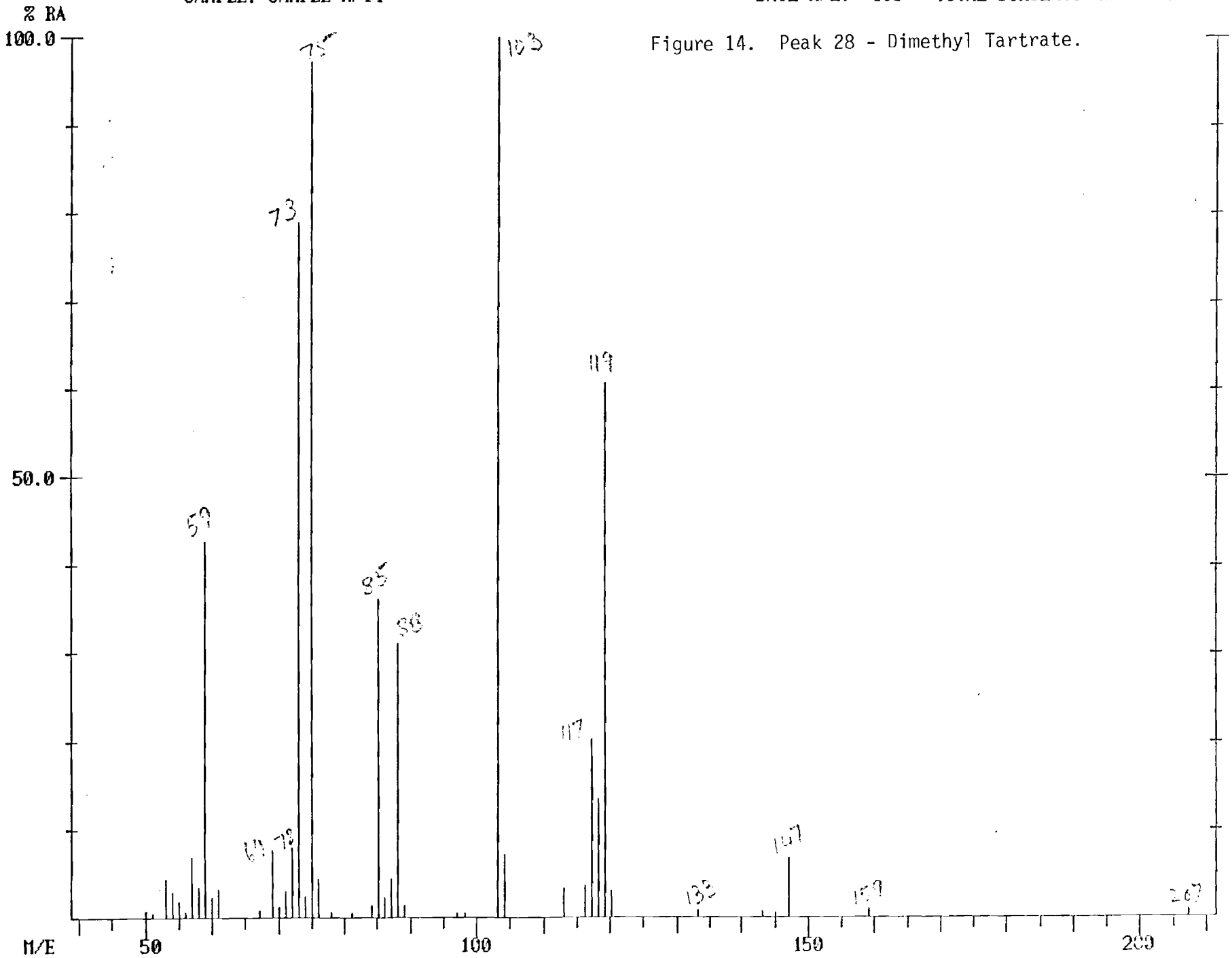


Figure 14. Peak 28 - Dimethyl Tartrate.

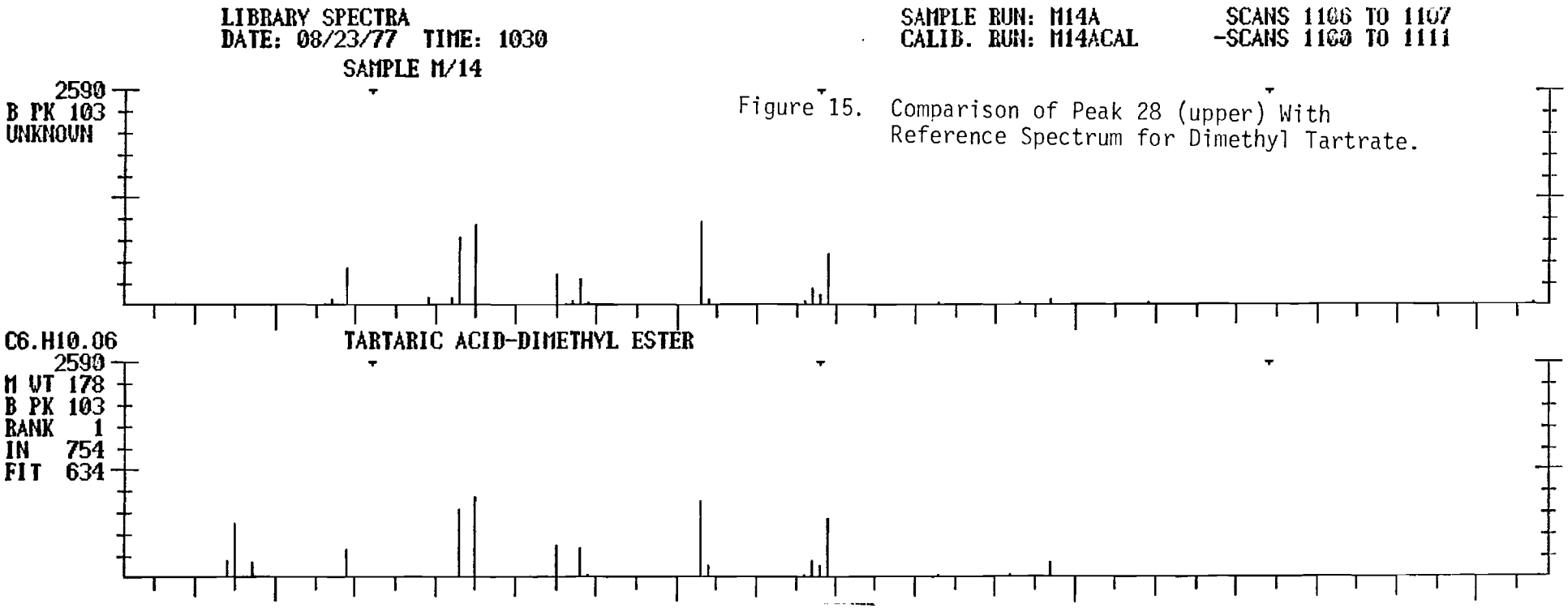
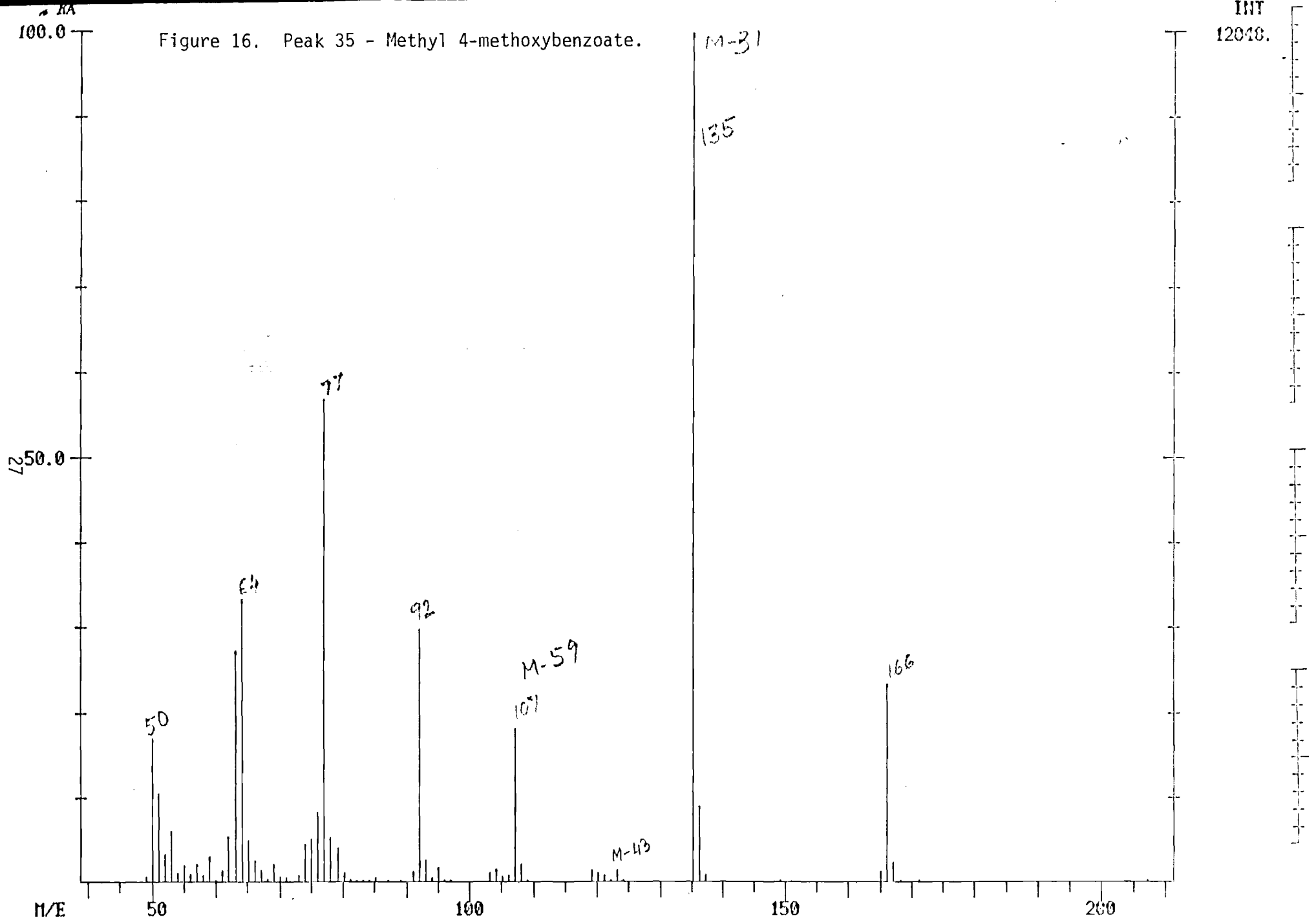


Figure 15. Comparison of Peak 28 (upper) With Reference Spectrum for Dimethyl Tartrate.

Figure 16. Peak 35 - Methyl 4-methoxybenzoate.



DATE: 08/23/77 TIME: 1030 SAMPLE RUN: M14A SCANS 1282 TO 1282
CALIB. RUN: M14ACAL

SAMPLE M/14

1717
B PK 135
UNKNOWN

Figure 17. Comparison of Peak 35 (upper) With Reference Spectra for 4- and 3- methoxybenzoates.

C9.H10.03 METHYL P-METHOXYBENZOATE
1717
M WT 166
B PK 135
RANK 1
IN 12511
FIT 717

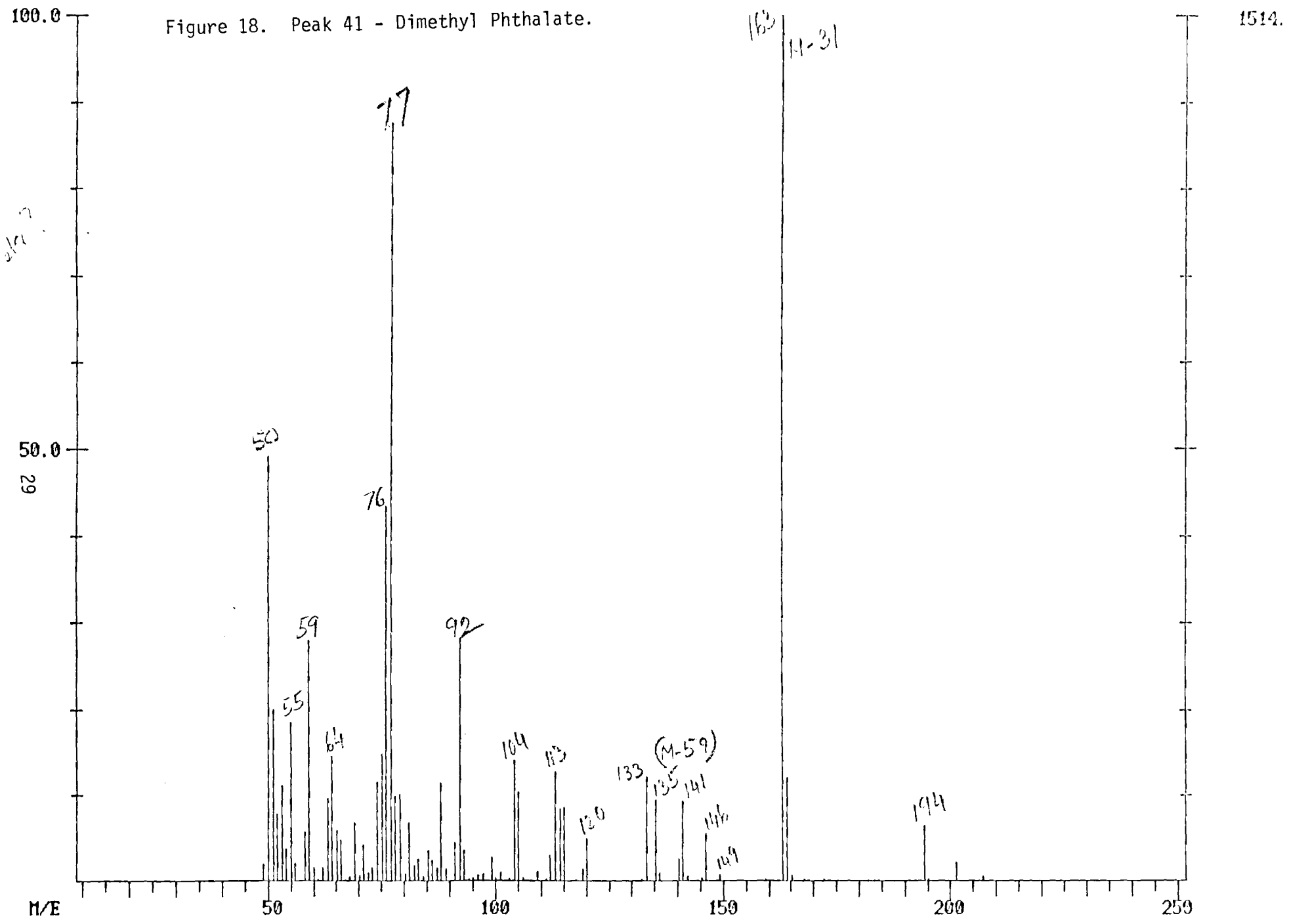
C9.H10.03 3-METHOXYBENZOIC ACID-METHYL ESTER
1717
M WT 166
B PK 135
RANK 2
IN 575
FIT 531

C9.H10.03 4-METHOXYBENZOIC ACID-METHYL ESTER
1717
M WT 166
B PK 135
RANK 3
IN 574
FIT 444

50 100 150 200
M/E

BASE M/E: 163 TOTAL IONIZATION: 10347.

Figure 18. Peak 41 - Dimethyl Phthalate.



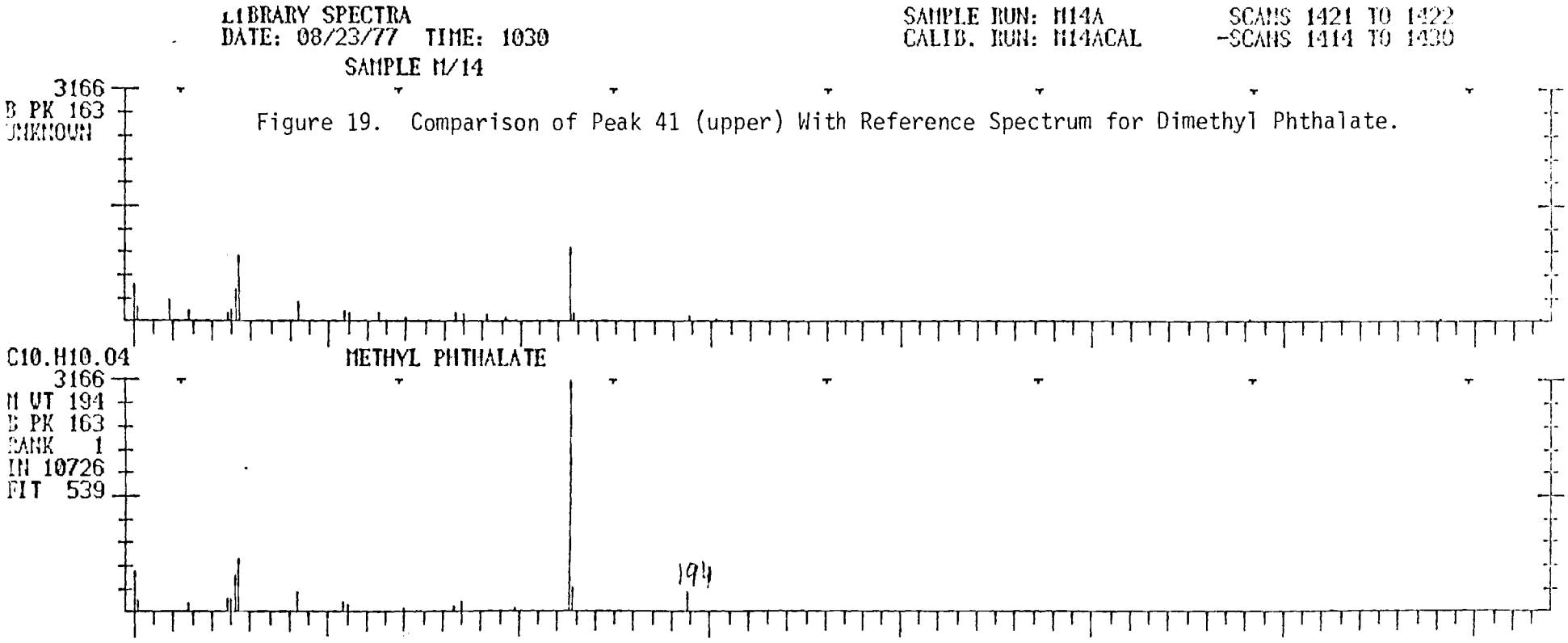


Figure 19. Comparison of Peak 41 (upper) With Reference Spectrum for Dimethyl Phthalate.

BASE M/E: 163 TOTAL IONIZATION: 2354.

INT
379.

Figure 20. Peak 43 - Dimethyl Terephthalate.

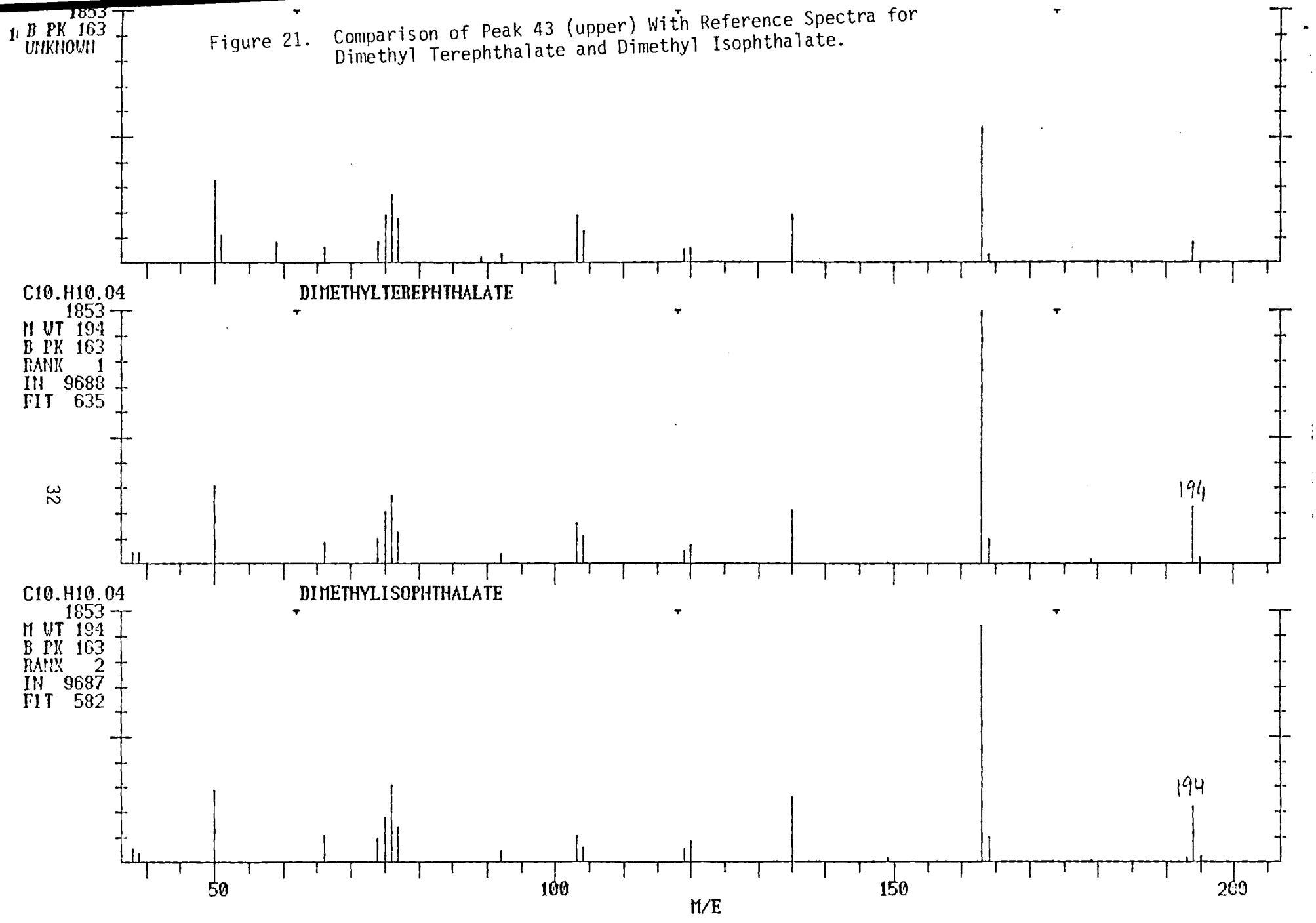


Figure 21. Comparison of Peak 43 (upper) With Reference Spectra for Dimethyl Terephthalate and Dimethyl Isophthalate.

SAMPLE: SAMPLE M/I4 BASE M/E: 163 TOTAL IONIZATION: 2186.

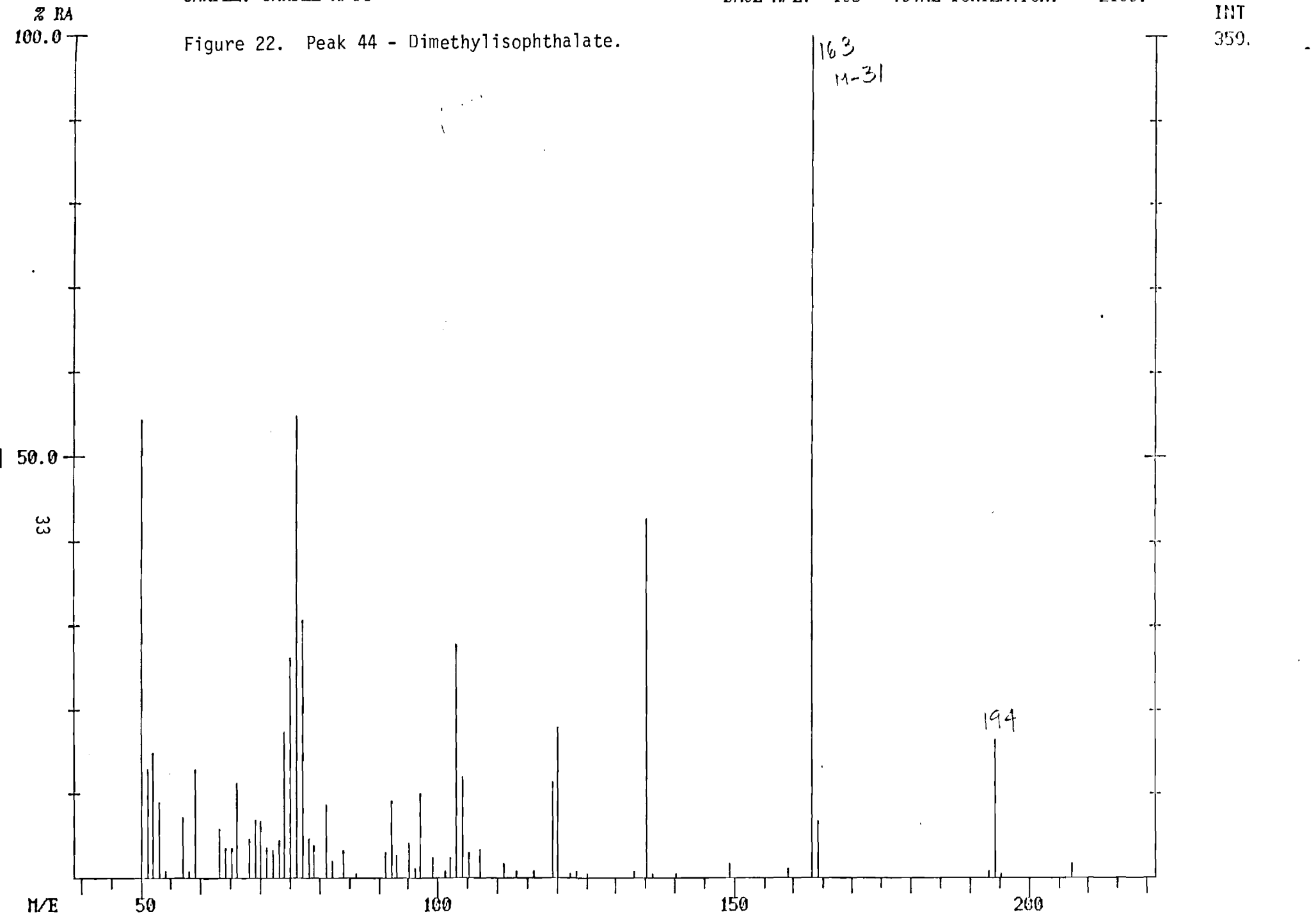


Figure 22. Peak 44 - Dimethylisophthalate.

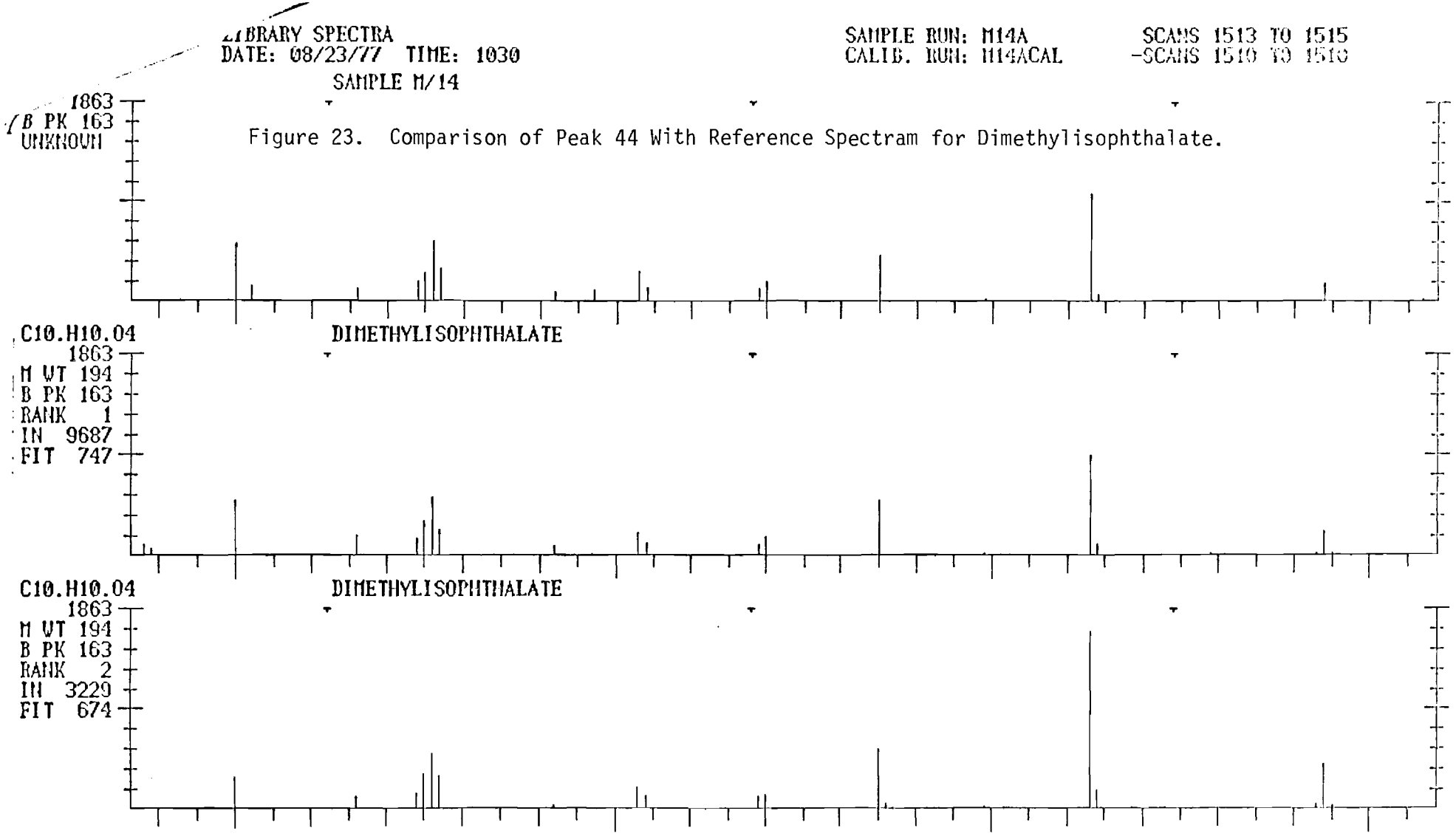


Figure 23. Comparison of Peak 44 With Reference Spectram for Dimethylisophthalate.

SAMPLE: SAMPLE 11/14 BASE M/E: 165 TOTAL IONIZATION: 4229.

Figure 24. Peak 54 - Methyl Dimethoxybenzoate Isomer.

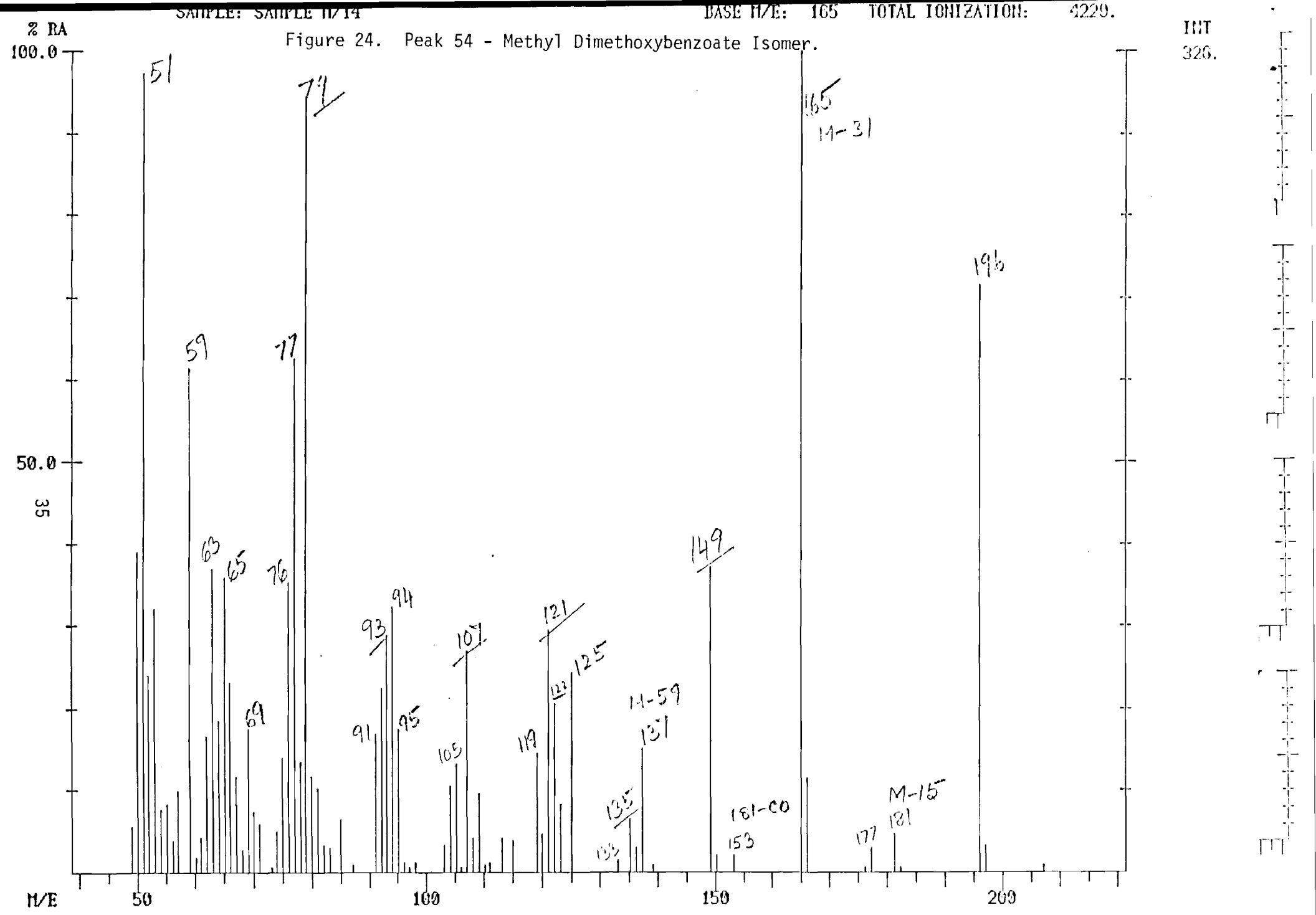


Figure 25. Peak 77 - Trimethylbenzene Tricarboxylate Isomer.

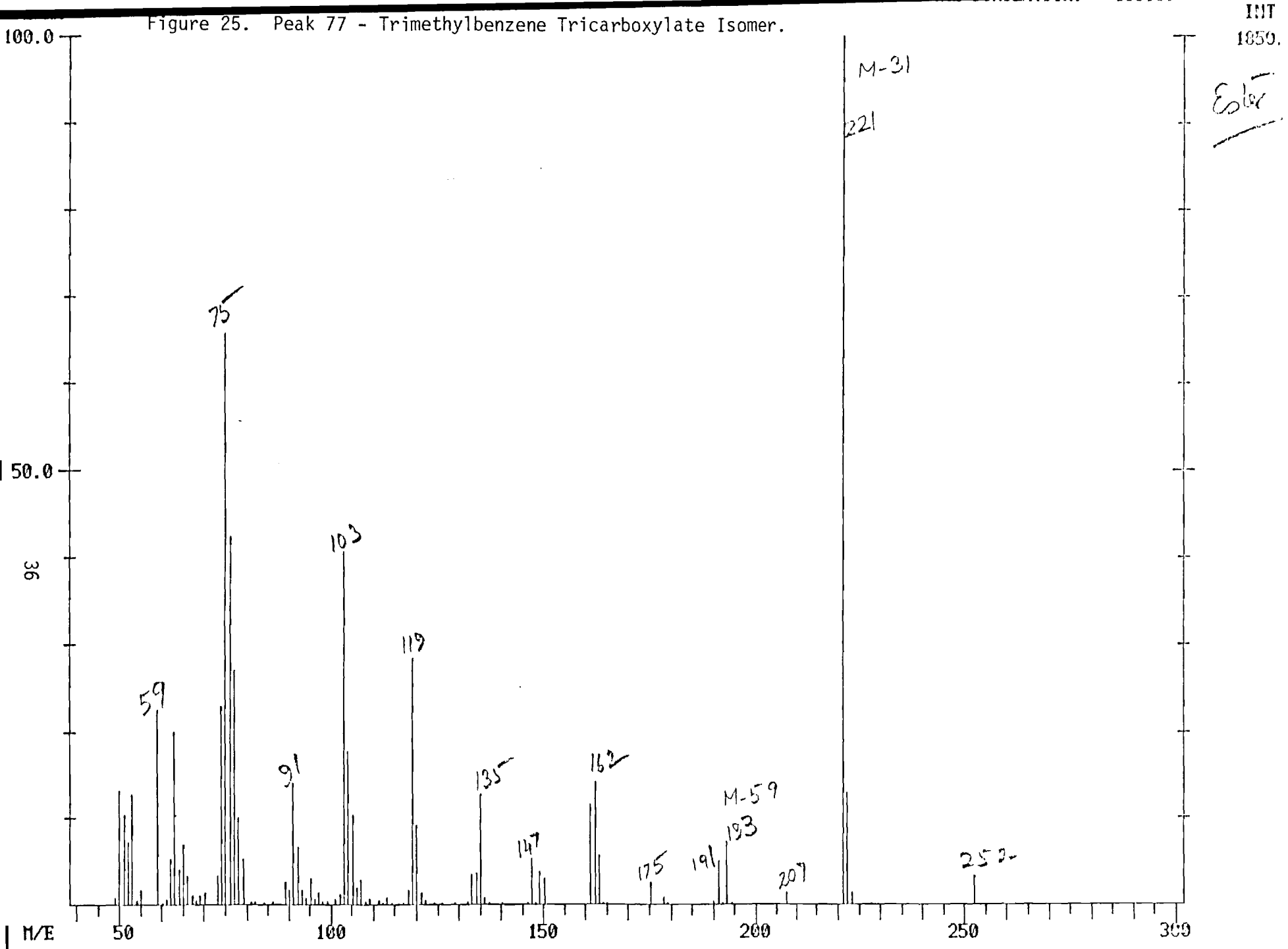
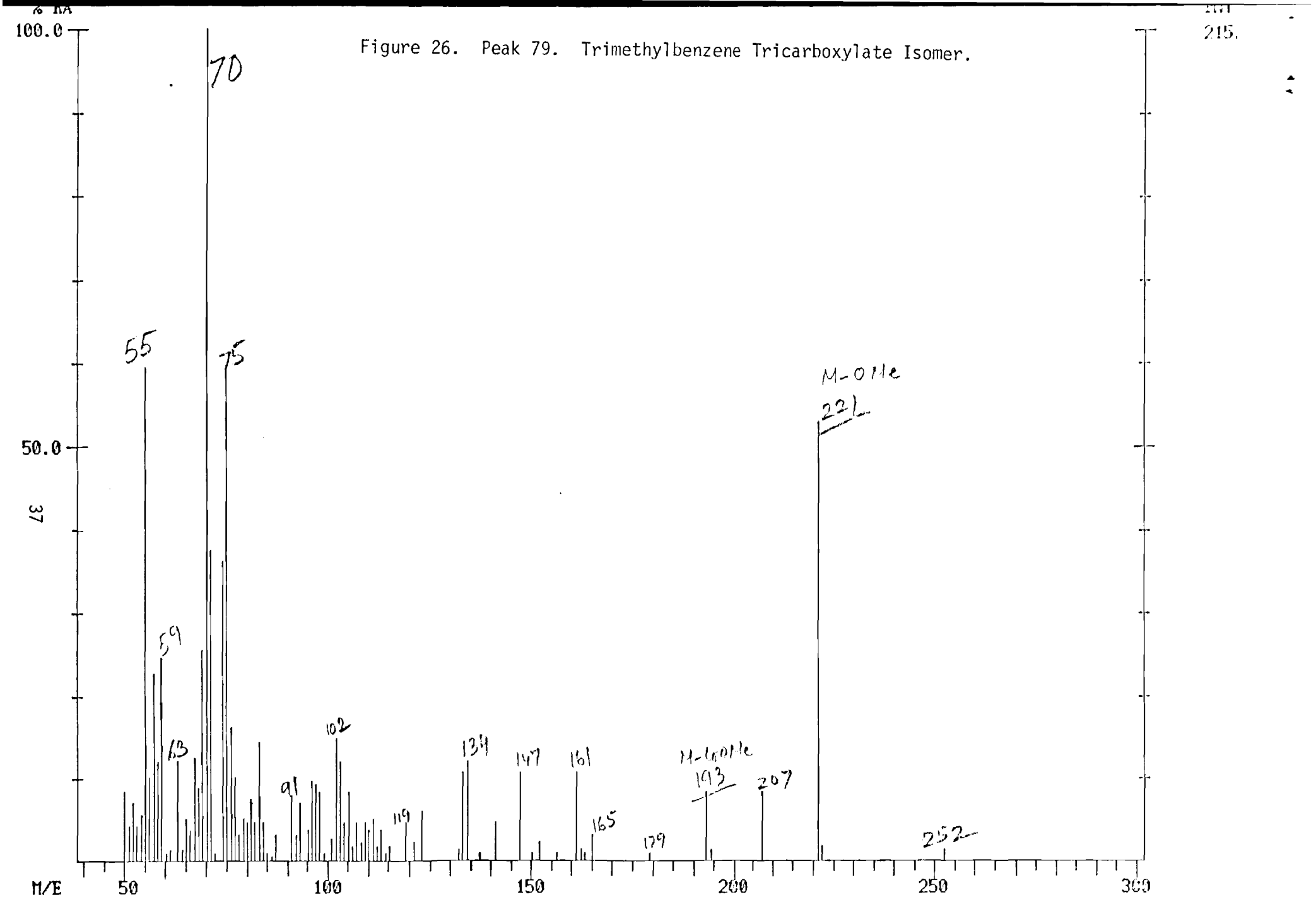


Figure 26. Peak 79. Trimethylbenzene Tricarboxylate Isomer.



BASE M/E: 57 TOTAL IONIZATION: 2110.

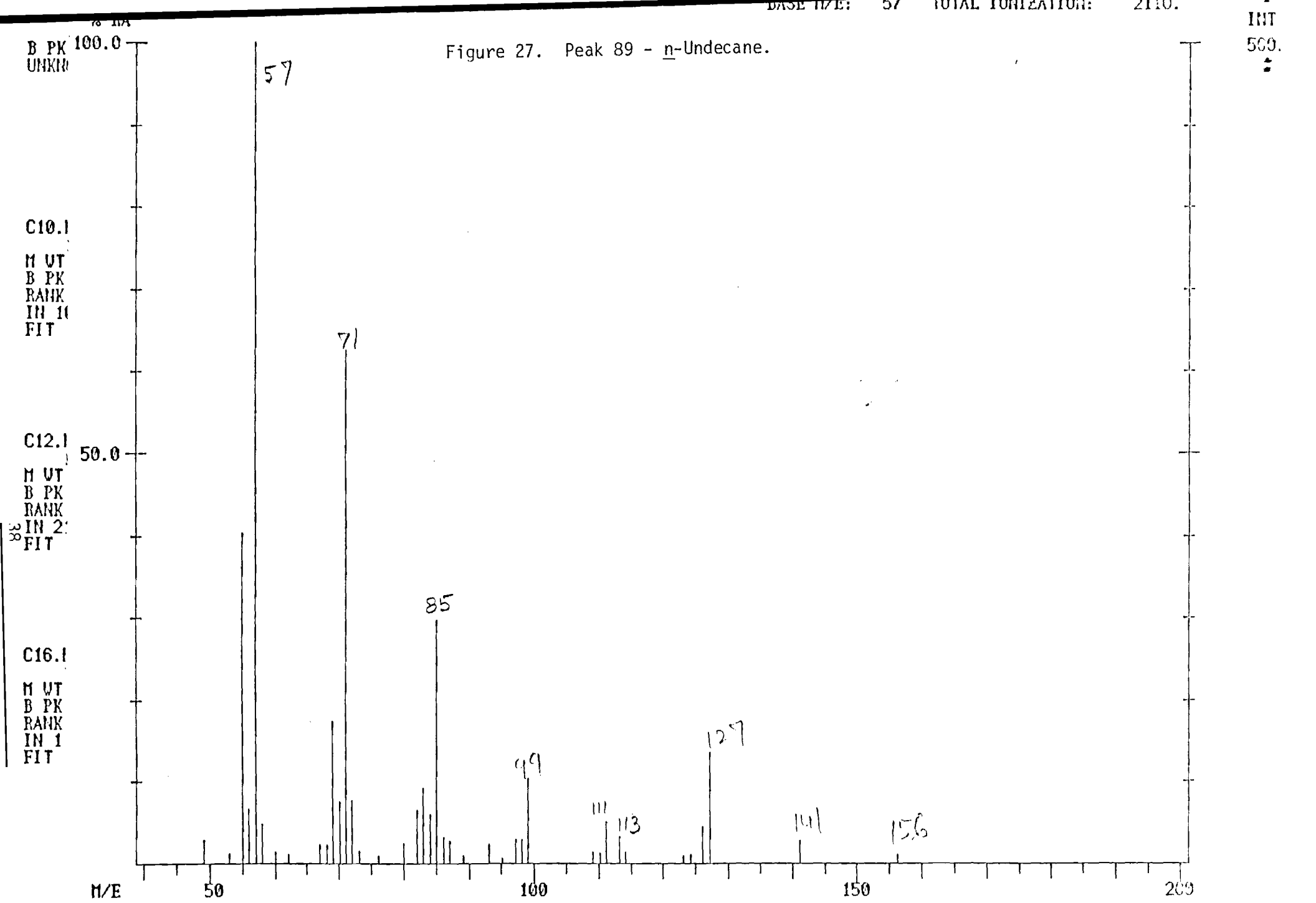
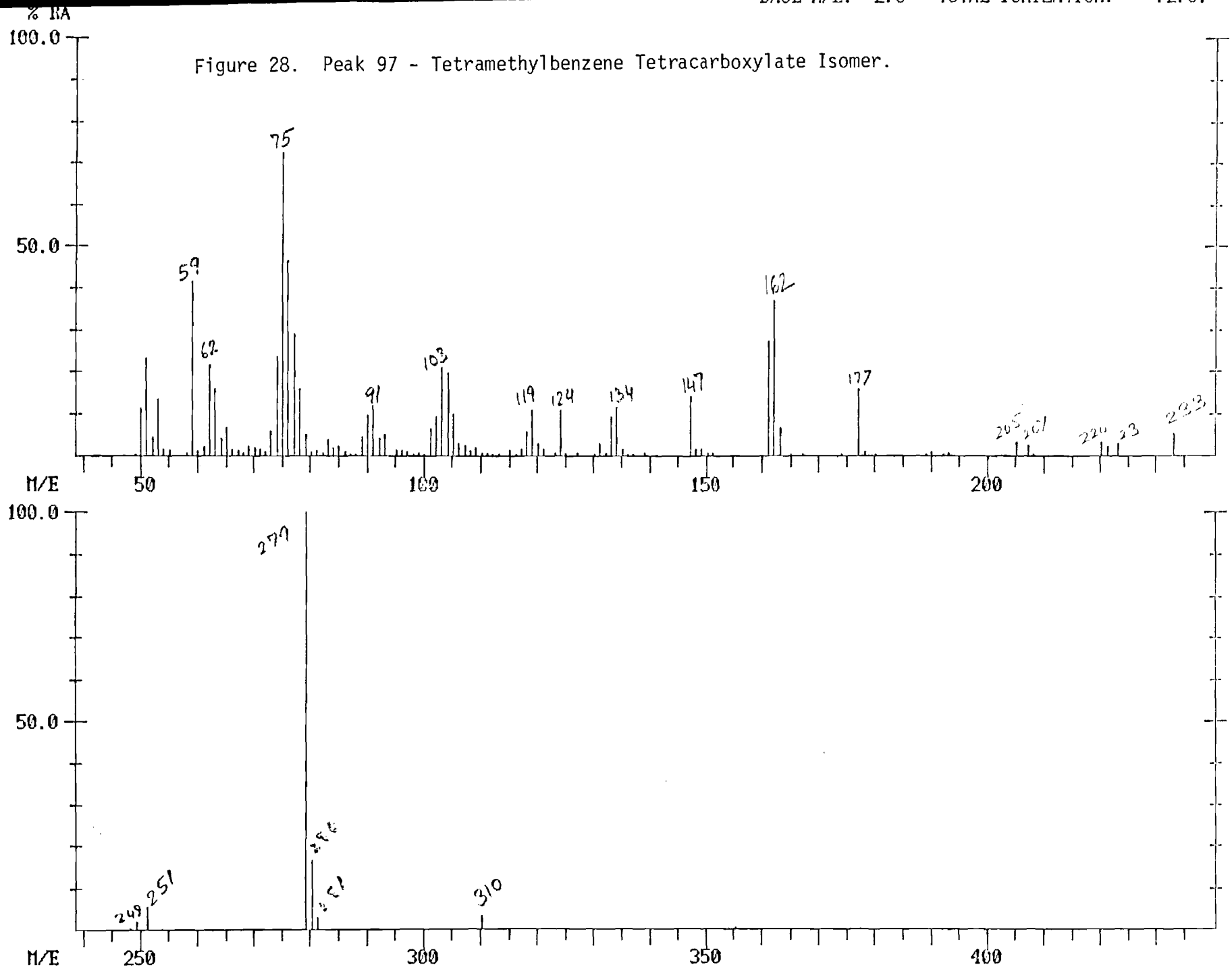


Figure 27. Peak 89 - n-Undecane.

Figure 28. Peak 97 - Tetramethylbenzene Tetracarboxylate Isomer.



E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report
December 5, 1977

by

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DISCLAIMER

This report has not been reviewed by the Criteria and Standards Division, Office of Water Supply, U. S. Environmental Protection Agency, and is intended for internal circulation only. The contents do not necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

I. PERSONNEL

No personnel changes have taken place during this reporting period.

II. EQUIPMENT

We continue the accumulation of practical experience with the Finnigan 4023 GC/MS system. Capillary columns with high resolution capabilities were used in our investigation of products from model compound - chlorine reactions. Experience has also been gained in the use of packed columns and in the chemical ionization mode of operation.

The mini-plants for investigation of water disinfection and purification effects on trace organic compound levels has been completely assembled and subjected to test runs. A 20-liter Pyrex bottle has been modified by addition of a tubulation and stopcock 4 cm from the bottom of the bottle. This modified bottle will serve as a reservoir for the water samples under study. The gravity flow from the reservoir can be controlled by a combination of head pressure and stopcock setting. The flow from the reservoir passes into a constant head overflow chamber and then to a mixing chamber consisting of a modified 500 ml Erlenmeyer flask. Chlorine and flocculant materials can be added to the mixing chamber. The treated water from the mixing chamber flows to a 3-liter capacity flocculating chamber. In the initial hydraulic testing of the mini-treatment plant, it was observed that cycling occurred with sufficient intensity to disrupt settling of flocculated material. The flocculating chamber has been redesigned and reconstructed; it has not been tested as yet. The reconstructed flocculating chamber will maintain the construction feature of the mini-treatment plant of having only Teflon or glass as the water contact materials.

III. GAS CHROMATOGRAPHIC STUDIES

The gas chromatographic studies completed during November were done with the GC/MS system. The GC conditions used for examination of samples from the factorial series of coniferyl alcohol-chlorine reaction were:

Column: 25 ml x 0.8 mm O.I. glass capillary

Stationary phase: SE 30

Carrier gas: Helium

Split/sweep ratio: 5.54

Transfer line temp.: 25^{o}

Ion source temp.: 25^{o} .

Temperature program: 40 to 190^{o} at 10^{o}/min; 1 min hold at 190^{o}

Figures 1 to 4 show total ion reconstructed chromatograms for 0.6 µl injections of distilled-in-glass methanol, distilled-in-glass benzene, a diazoethane reagent blank containing no coniferyl alcohol, and a sample of ethylated coniferyl alcohol (presumed), respectively. The similarity of the chromatograms in Figures 3 and 4 was the first indication of the uncertain identity of the presumed coniferyl alcohol used in the factorial experiments. The peaks at scans 640 and 660 were due to butylated hydroxytoluene isomers (BHT). These peaks were seen in a wide variety of injections of solvents and factorial series samples and even in an o-phenyl phenol sample. We now ascribe the regular appearance of BHT peaks during this period of operation to a contaminated injection port since they disappear after cleaning and use of a fresh septum.

IV. ISOLATION OF AQUATIC HUMIC MATTER

The procedure for isolation of aquatic humic matter from Satilla River water has been standardized in the course of the processing of 471 l of sample. The following operations are done in the sequence shown:

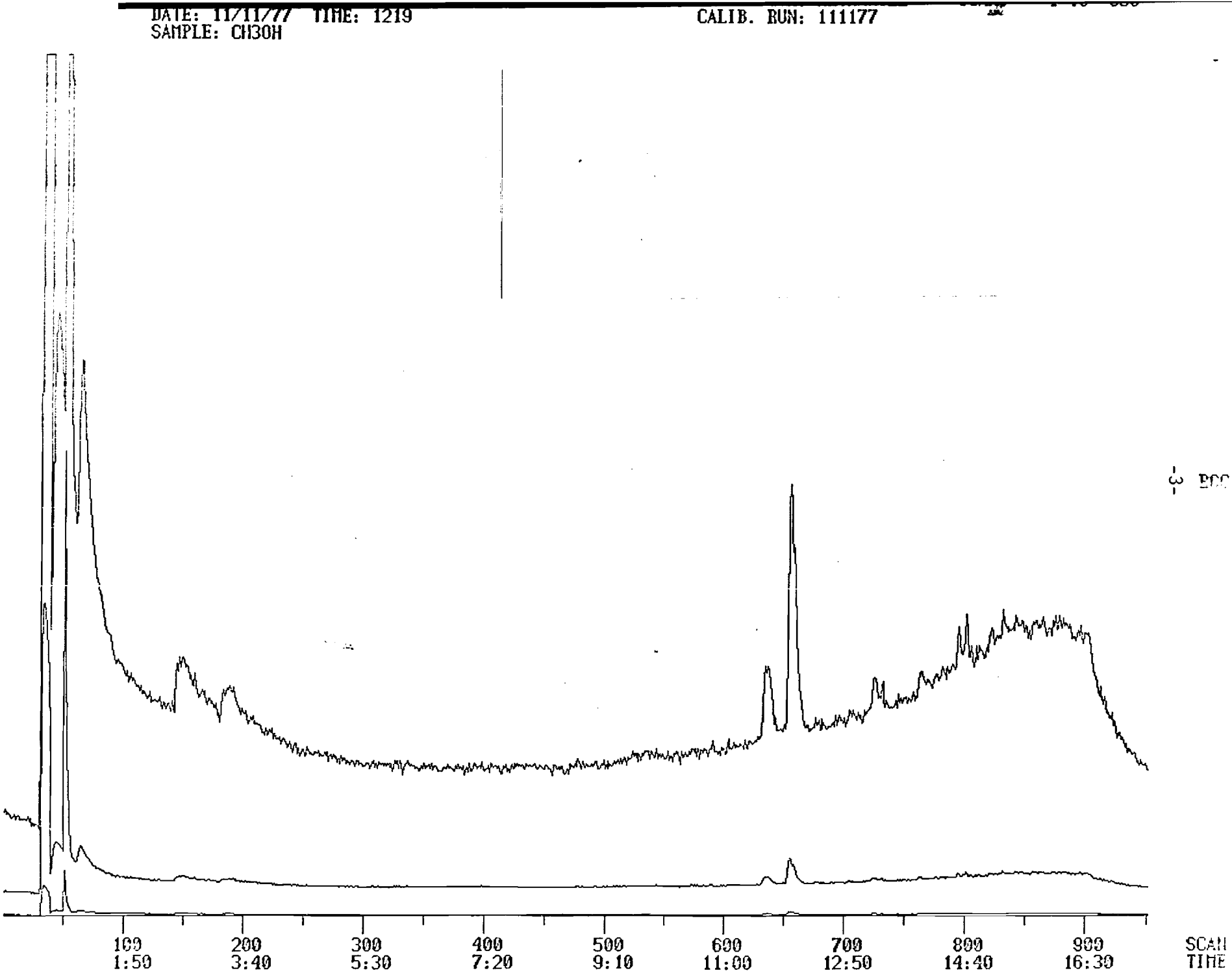

Figure 1. Reconstructed Total Ion Chromatogram from Injection of Purified Methanol.

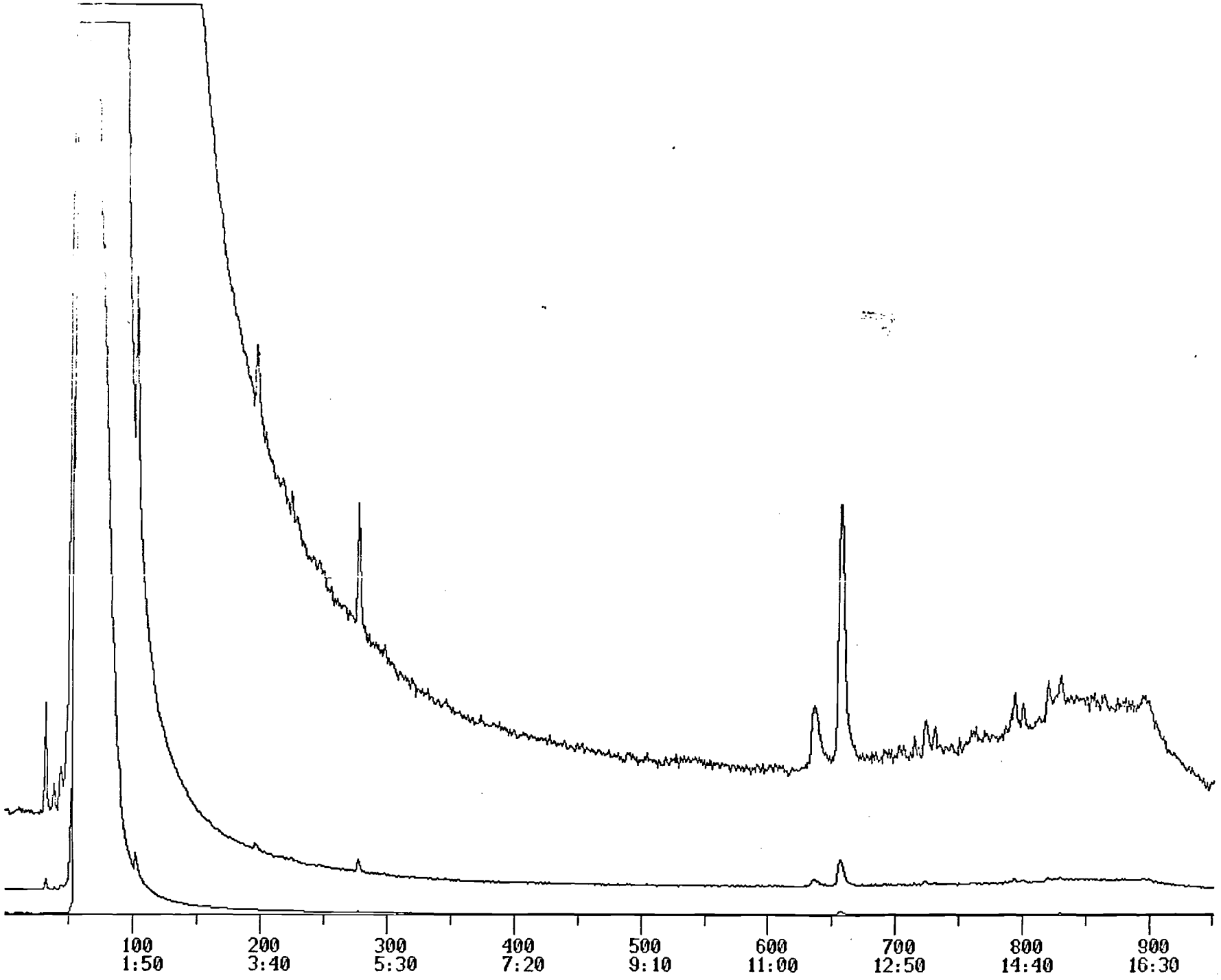

Figure 2. Reconstructed Total Ion Chromatogram from Injection of Purified Benzene.

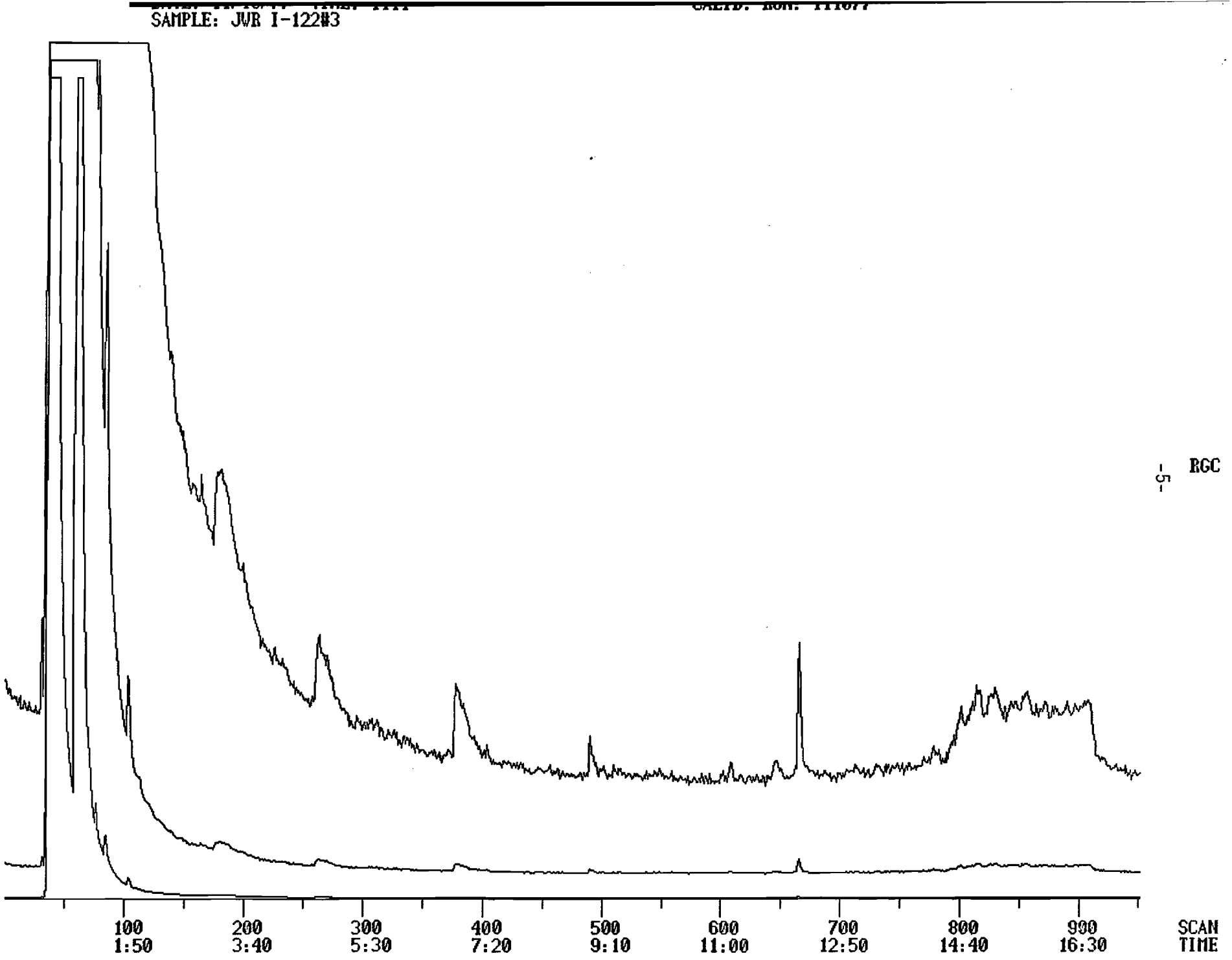

Figure 3. Reconstructed Total Ion Chromatogram from Injection of Diazoethane Reagent Blank.

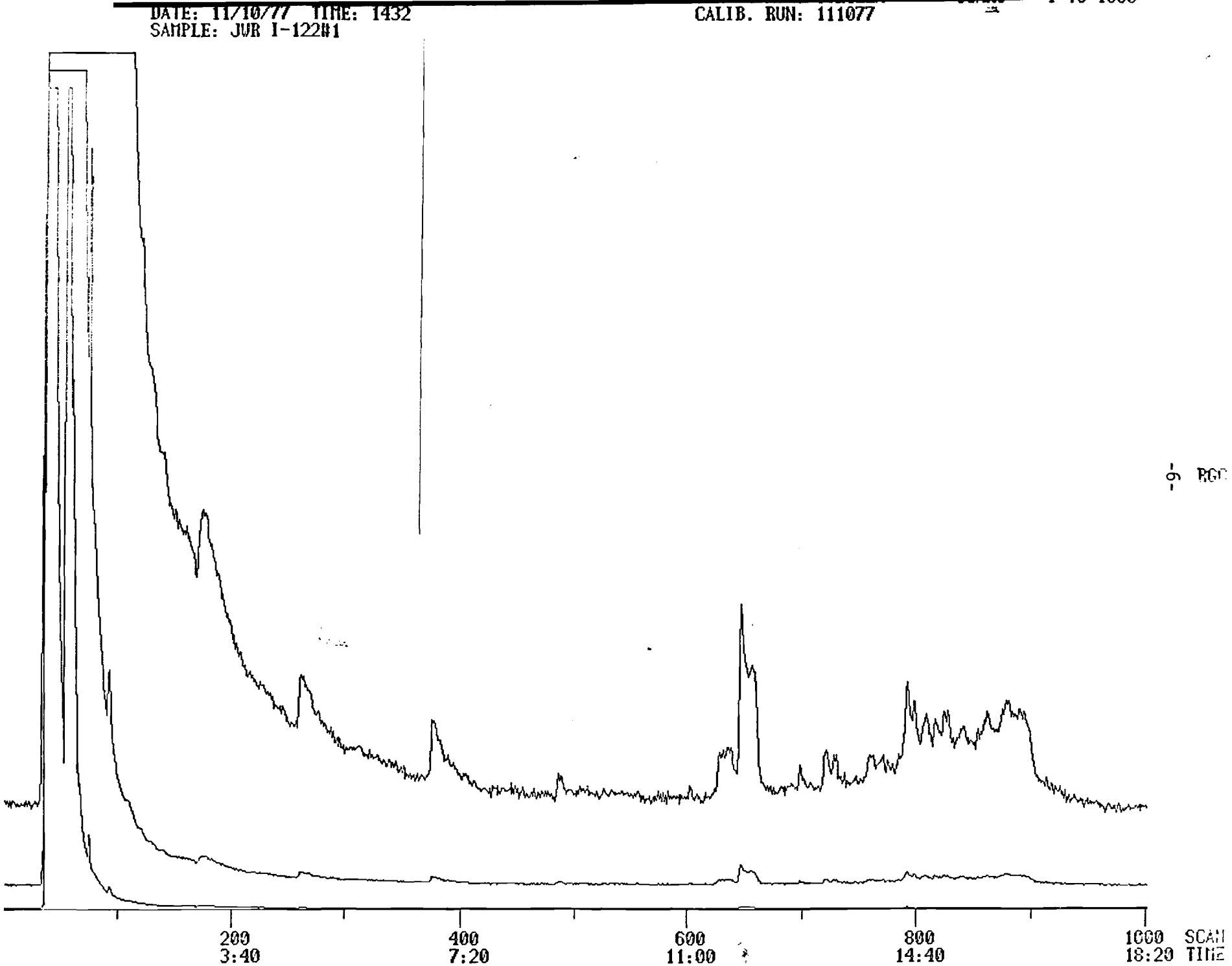

Figure 4. Reconstructed Total Ion Chromatogram from Injection of Pressured Coniferyl Alcohol Reaction with Diazoethane.

1. Clarification of River Water - The best results are obtained by centrifugation at 17000 R.P.M. with a residence time of 1 min., and a flow rate of 400 ml/min. Alternatively, the river water can be filtered through glass wool plugs which are changed after each 10 l of unfiltered water. These filtrates show more clogging of the resin columns than do the centrifuged river water.
2. Acidification - Addition of 12 N hydrochloric aicd to pH 1.7.
3. Concentration - Passing through Aberlite XAD-7 resin.
4. Elution - Solution of 33 ml of purified triethylamine/l water.
5. Removal of excess triethylamine-Rotary evaporator at reduced pressure.
6. Triethylamine Hydrochloride Removal - Continuous extraction of the concentrated aqueous humic matter with chloroform for 24 hours. Some yellow material is also removed.
7. pH Adjustment - Shaking the aqueous solution with cationic exchanger until a constant pH is reached.
8. Freeze Drying - The dry product was equivalent to a concentration of 66 mg/l of the original river water. The elemental analysis gave: C, 50.13; H, 3.50; N, 0.80; ash 0.00%.

A sample which was freeze dried after Step 6 had the empirical analysis: C, 53.05; H, 6.02; N, 3.79; ash 0.00%.

The infrared spectra of freeze dried samples of processed Satilla River water are shown in Figure 5 (after Step 6) and Figure 6 (after Step 7).

V. OZONIZATION OF METHYLATED AQUATIC HUMICS

A sample of aquatic humics (A-3) was methylation with diazomethane. The product (0.6 g) was suspended in 50 ml of dichloromethane, filtered, cooled in

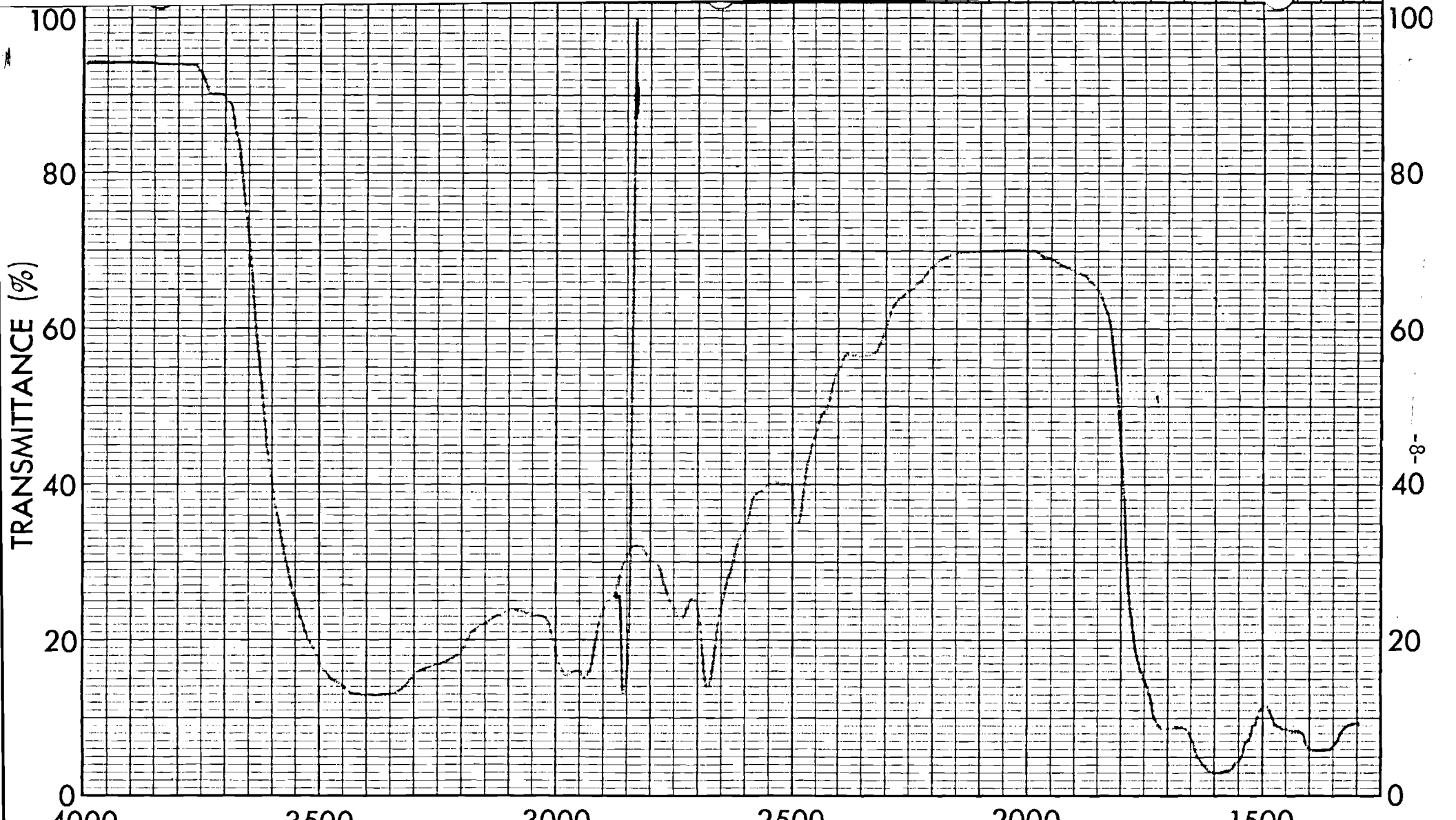

Figure 5. Infrared Spectrum of Freeze Dried Concentrated Aqueous Humic Matter from Satilla River Water Samples (KBr Pellet) After Step 6. (Continued on next page.)

SAMPLE M/26 Aq. humics after step 6.	CURVE NO.	SCAN SPEED	OPERATOR M. Ghosal
	CONC.	SLIT	DATE 11-9-77
ORIGIN	CELL PATH	REMARKS	
SOLVENT KBr	REFERENCE		

PART NO. 237-1024

PERKIN ELMER

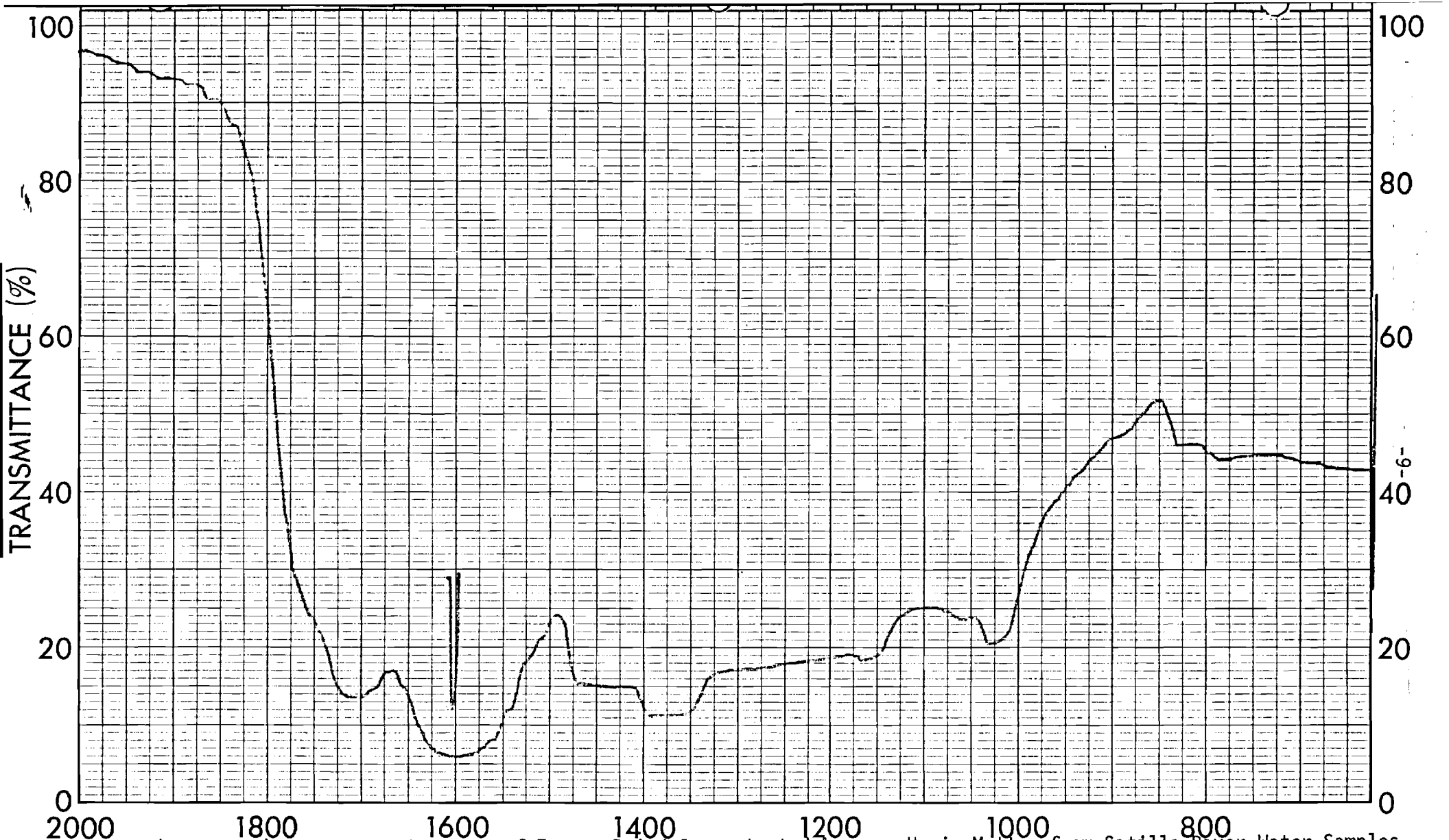

Figure 5 (Continued). Infrared Spectrum of Freeze Dried Concentrated Aqueous Humic Matter from Satilla River Water Samples (KBr Pellet) After Step 6.

SAMPLE M/26 Aq. humics aftr extn. with $CHCl_3$	CURVE NO.	SCAN SPEED	OPERATOR M. Ghosal
ORIGIN	CONC.	SLIT	DATE 11-9-77
SOLVENT KBr	CELL PATH	REMARKS Contains Et_3NH^+ salts	
	REFERENCE		

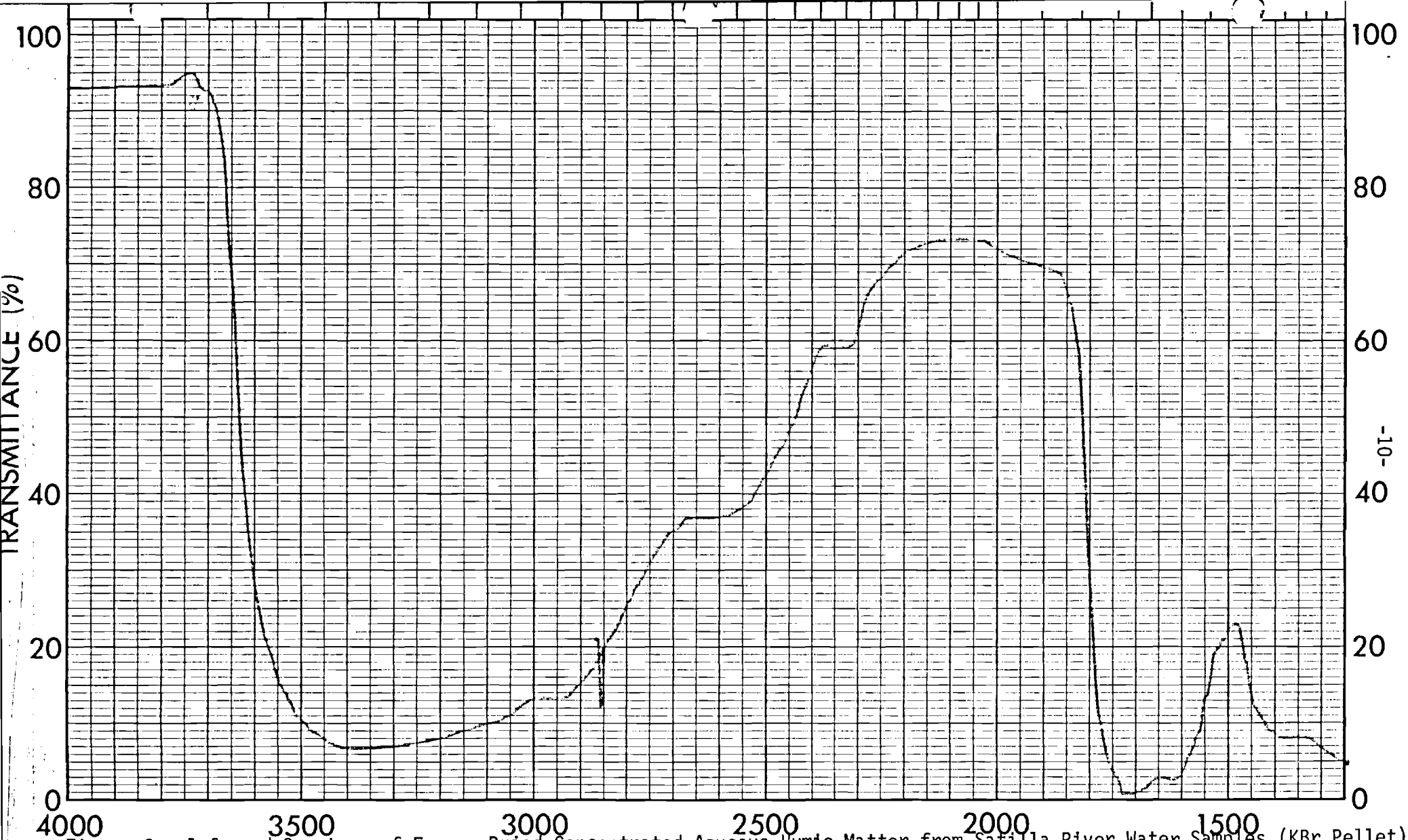

Figure 6. Infrared Spectrum of Freeze Dried Concentrated Aqueous Humic Matter from Satilla River Water Samples (KBr Pellet) After Step 7. (Continued on next page.)

SAMPLE M/28 Desalted aq. humics	CURVE NO.	SCAN SPEED	OPERATOR M. Ghosal
	CONC.	SLIT	DATE 11-9-77
ORIGIN	CELL PATH	REMARKS	
SOLVENT KBr	REFERENCE		

PERKIN ELMER

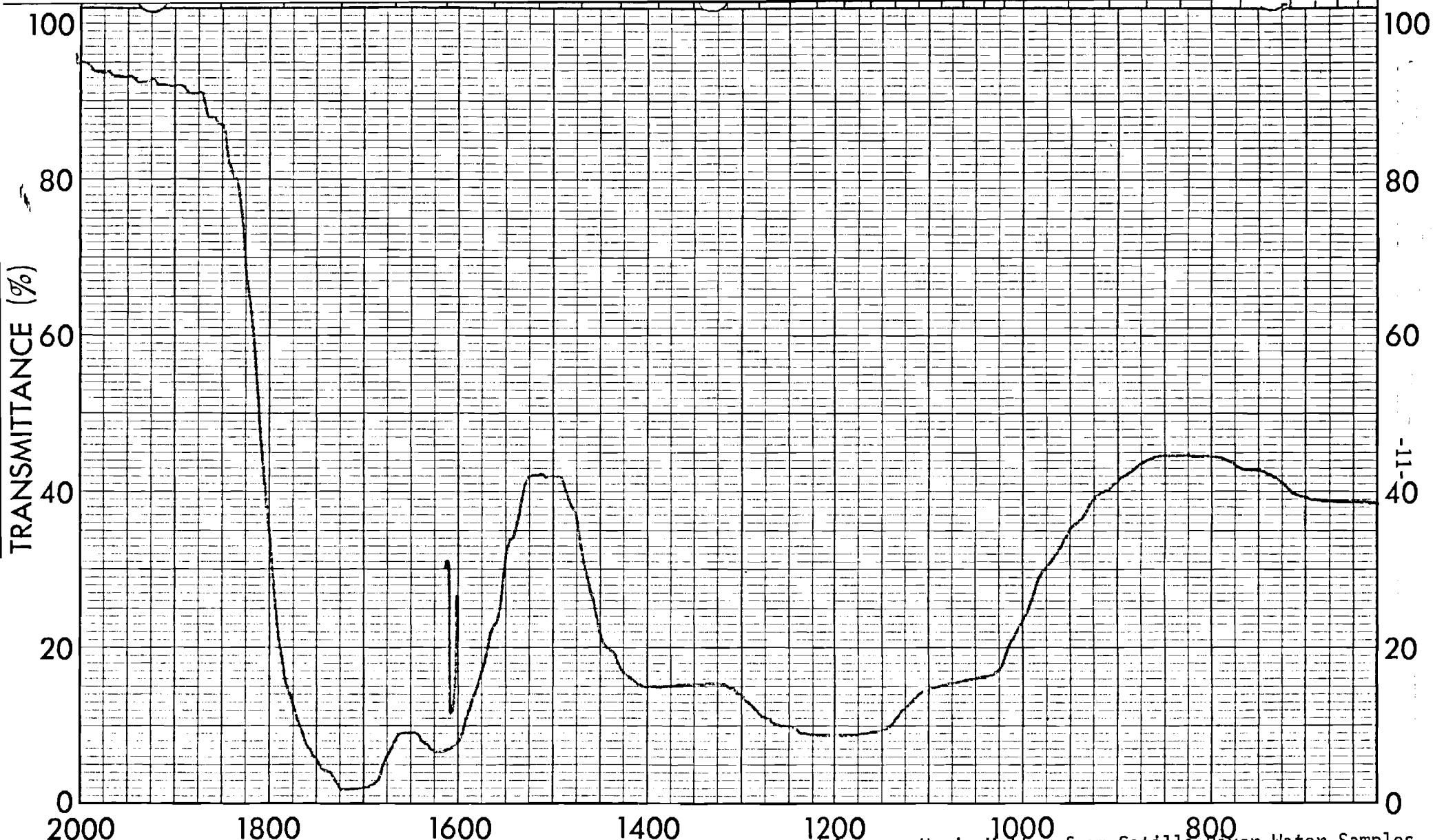

Figure 6 (Continued). Infrared Spectrum of Freeze Dried Concentrated Aqueous Humic Matter from Satilla River Water Samples (KBr Pellet) After Step 7.

SAMPLE M/28 Desalted aq. humics.	CURVE NO.	SCAN SPEED	OPERATOR M. Ghosal
	CONC.	SLIT	DATE 11-9-77
ORIGIN	CELL PATH	REMARKS	
SOLVENT KBr	REFERENCE		

ice-water, and treated with an excess of ozone. After reacting with water and zinc dust overnight, the oxidation product was isolated.

The analysis of the oxidation product was accomplished using GC/MS under the following operational conditions:

Column: 6' x 2 mm ID glass

Stationary phase: 3% OV-1 on 60/80 mesh gas chromatograph Q

Carrier gas: He at 30 ml/min

Temperature program: isothermal at 220° for 1 min, increase at 5°/min to 260° and hold for 3 min.

Injector, transfer line, and ion source: all at 250°C

The reconstructed total ion chromatogram (with the temperature program stopped at approximately scan #690) is shown in Figure 7. The electron impact mass spectra of the scan at scan #310 is shown in Figure 8. The spectrum has intensities increased by a factor of 10 above m/e 200. The chemical ionization mass spectra of scan #321 (from a different injection but corresponding to scan #310) is shown in Figure 9.

The library search for the identity of the compound in the peak at scan #310 shows a moderately good fit for dioctylphthalate as shown in Figure 10.

Khan and Schnitzer have reported[1] the isolation of dioctylphthalate after oxidation of fulvic acid. Ogner and Schnitzer[2,3] have suggested that dioctylphthalate has a biosynthetic origin. We are certain that the dioctylphthalate must have come from the sample and not from contamination; we agree with Schnitzer, et al. on this point. The present evidence suggests that dioctylphthalate (or diisooctylphthalate) may have been trapped in the complex humic structure and released after oxidation. Work is continuing to identify the other products of the ozonization reaction.

---

1. S.U. Khan and M. Schnitzer, Canadian J. Chem., 49, 2302 (1971).
2. M. Schnitzer and G. Ogner, Israel J. Chem., 8, 505 (1970).
3. G. Ogner and M. Schnitzer, Sci, 170, 317 (1970).

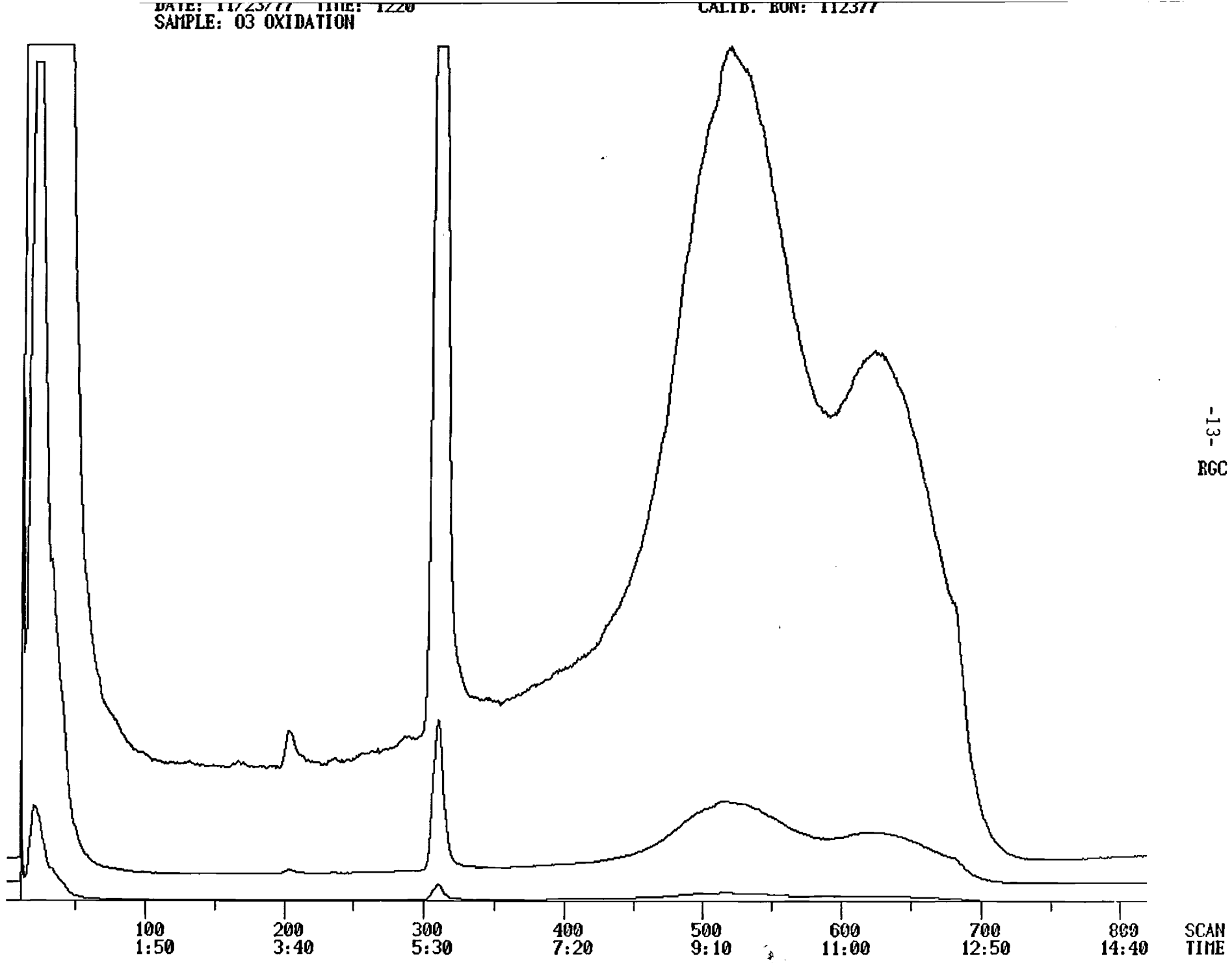

Figure 7. Reconstructed Total Ion Chromatogram of Separation of Oxidation Products of Methylated Aquatic Humic Matter.

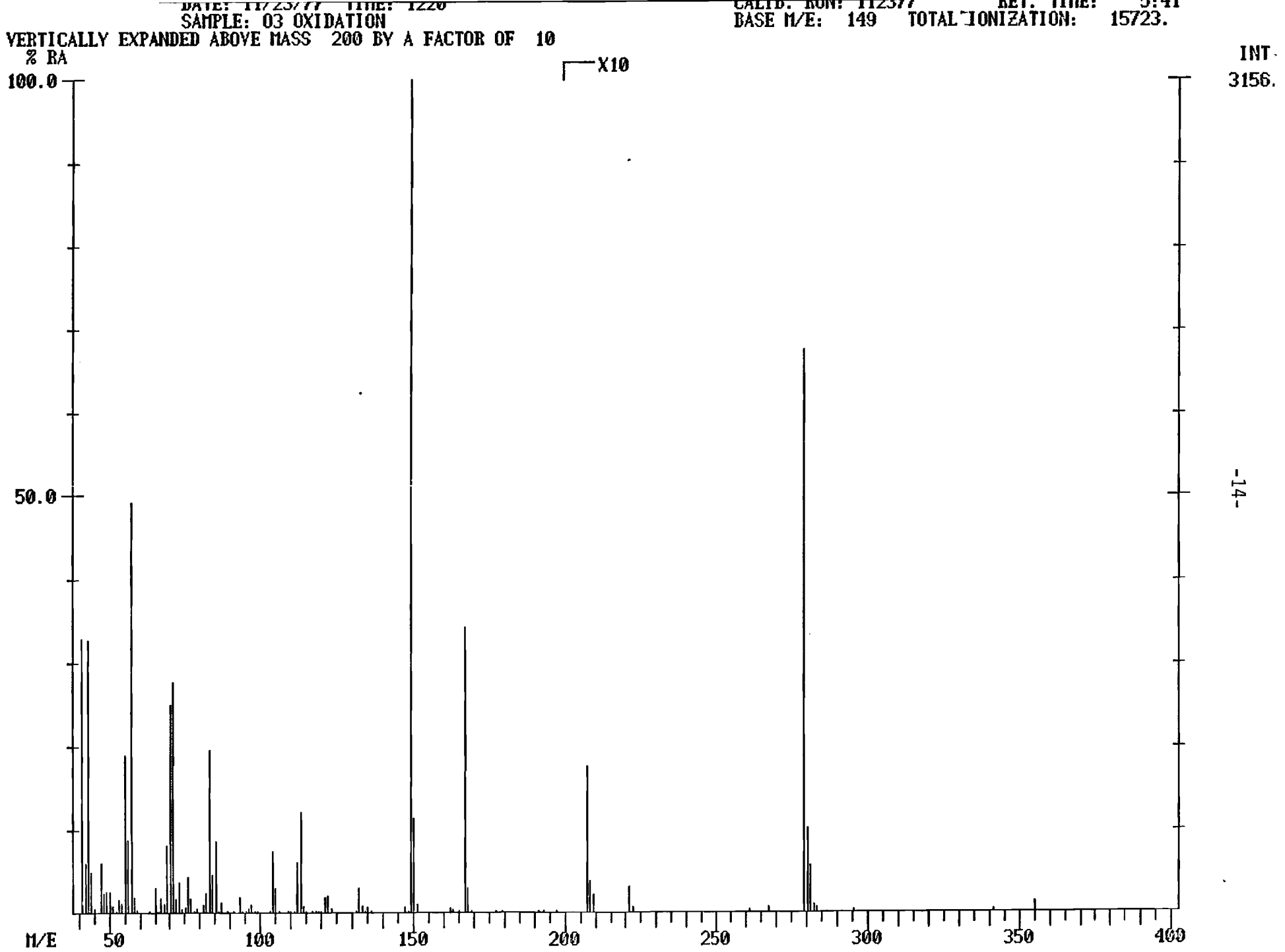

Figure 8. Electron Impact Mass Spectra of Scan #310 of Figure 7.

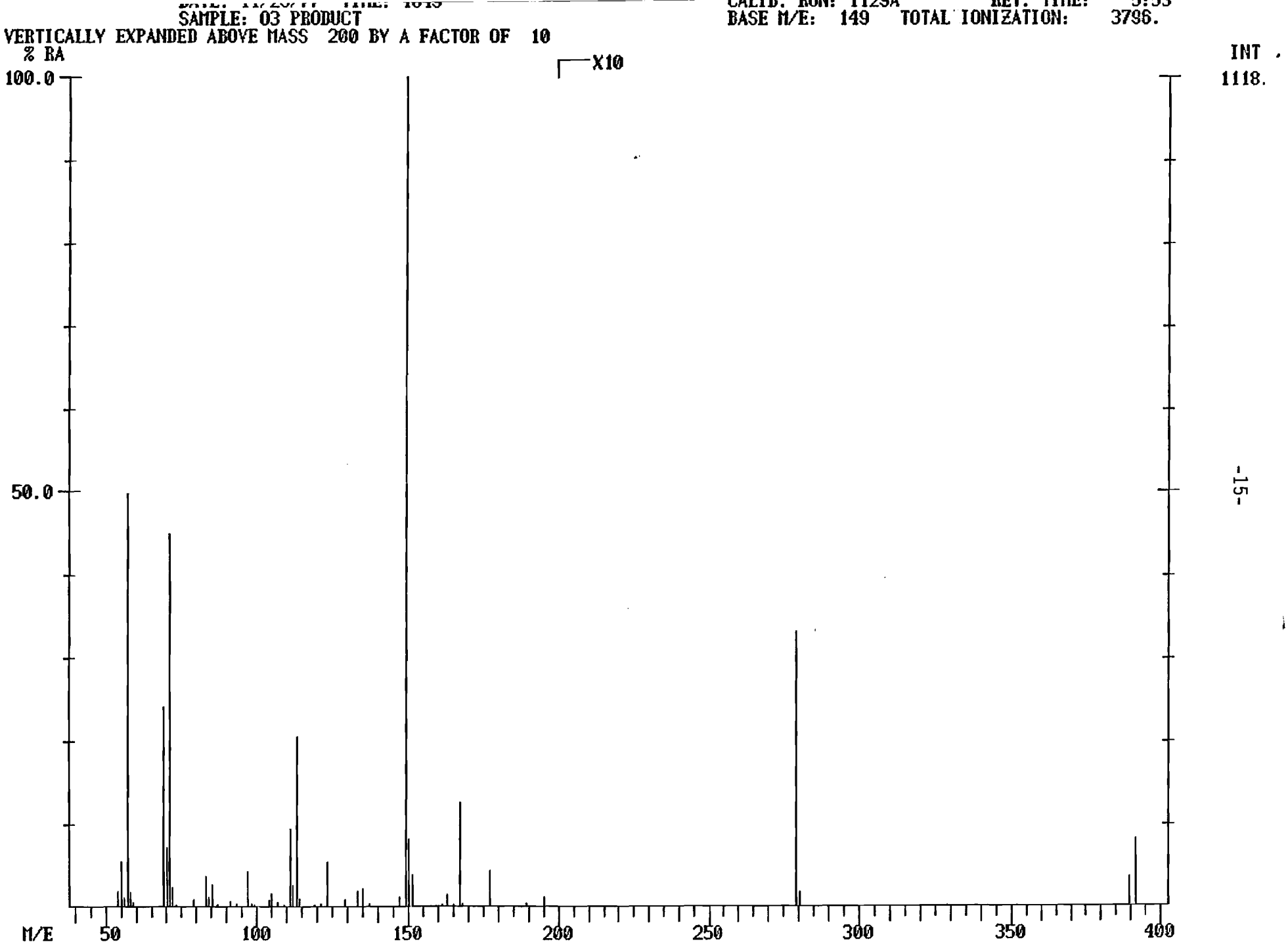

Figure 9. Chemical Ionization Mass Spectra of Peak Corresponding to Scan #310 of Figure 7.

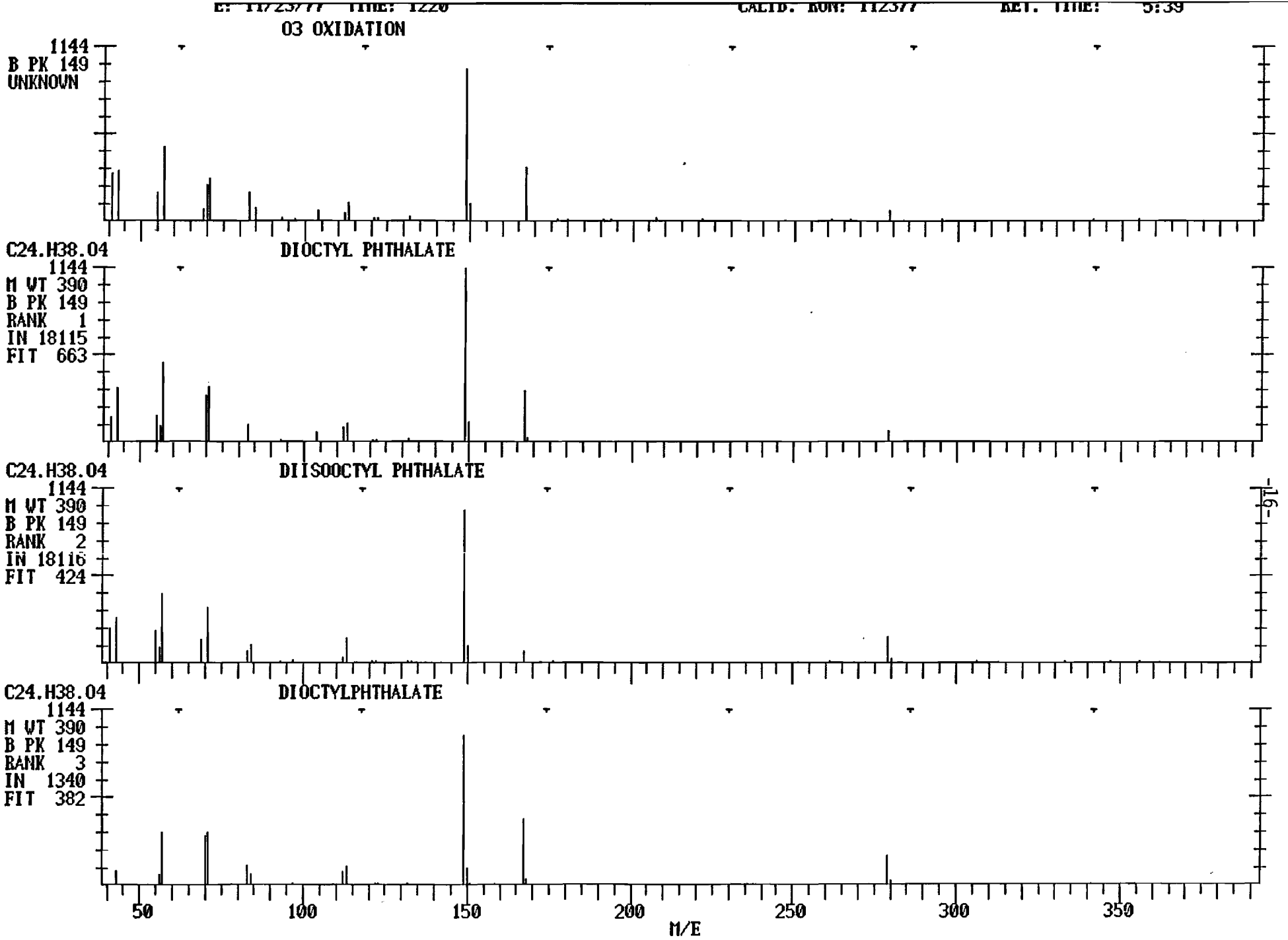

Figure 10. Results of Computer Library Search for Identity of Compound in Peak at Scan #309 (see Figure 7).

VI. MODEL COMPOUND SERIES - SERIES I, FACTORIAL EXPERIMENT

A serious setback occurred in this part of our studies when it was found that the presumed high purity coniferyl alcohol used in a large number of model reactions with chlorine, chlorine dioxide and ozone was a different, and as yet unidentified, material.

The identity of the presumed coniferyl alcohol became suspect when no evidence was found for coniferyl alcohol ethyl ether in factorial series concentrates analyzed by capillary column GC/MS. Direct treatment of a suspension of presumed coniferyl alcohol (4.9 mg) in ether-benzene-methanol with excess diazoethane in pentane did not cause suspended material to react. The excess diazoethane was removed by gentle boiling and evaporation of the suspension to a volume of 15 ml. The solvent volume was adjusted to 50 ml with pentane. Injection of 1 μl of the supernatant liquid of the suspension into a capillary column GC/MS system gave no evidence for coniferyl alcohol ethyl ether or any other compound related to coniferyl alcohol.

Direct examination of the presumed coniferyl alcohol (purchased from Pfaltz and Bauer, Inc., Stamford, Connecticut) led to a wide difference among observed and reported properties as tabulated in Table 1. The presumed coniferyl alcohol was not soluble enough in chloroform to give an infrared absorption spectrum in a 0.025 mm sodium chloride liquid sample cell. An infrared spectrum of the presumed coniferyl alcohol mixed with potassium bromide and pressed into a pellet showed broad absorption bands, usually characteristic of a mixture of related compounds, and did not match the partial (5.5 - 10.5μ) spectrum repoted.[4] Communication with the supplier of the presumed coniferyl alcohol has not, as yet,

4. Allen and Byers, J. Amer. Chem. Soc. 71, 2683 (1949).

Table 1

Reported and Observed Properties for Coniferyl Alcohol and Presumed Coniferyl Alcohol

Property	Coniferyl Alcohol	Presumed Confieryl Alcohol
Melting Point	73-74° (5)	> 200°
	72-73° (6)	
Solubility		
Water, Cold	Insoluble (6)	Soluble
Ethanol	Soluble (6)	Insoluble
Ethyl Ether	Soluble (6)	Insoluble
Ferric Chlorine Test	Positive (6)	Negative

5. Allen and Byers, op. cit., 2683.
6. Tiemann and Harrmann, Ber. 7, 611(1874).

provided an explanation of the composition of the material delivered. Our speculation is that the presumed coniferyl alcohol is a polymerized transformation product of coniferyl alcohol due to the literature[4] statement "coniferyl alcohol slowly decomposes on standing and formed polymeric material with exceeding ease."

Further work using model compounds in the factorial series must use authentic coniferyl alcohol (prepared just prior to treatment by the emulsin catalyzed hydrolysis of coniferin), or more stable model compounds yet to be selected.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

January 6, 1978

by

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Atlanta, Georgia 30332

68-01-4480

Dr. Charles Trichilo
Criteria and Standards Division
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*Principal Investigator to whom inquiries should be directed.

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PERSONNEL

No personnel changes have taken place during this reporting period.

EQUIPMENT

The modified mini-pilot facility for the controlled treatment of water under conditions which simulate the production of potable water has been reassembled. Hydraulic tests using tap water and flocculated raw water have confirmed that the problem of cycling in the flocculation chamber has been eliminated. The test facility is therefore ready for use with model compounds and various raw water samples. A photograph of the apparatus is shown in Figure 1. The post-treatment reservoir (clear well) and mixing chamber for final pH and chlorine residual adjustment are not shown. After shakedown runs with resorcinol, the apparatus will be used in a series of more comprehensive tests which will be discussed in another section of this report.

We continue to have problems with our LC detection system. The manufacturer's representative now feels that he has isolated the problem so that we can bring this valuable tool back on-stream so that it can be used for the analysis of non-volatile halo-organics.

A new software package for the GC/MS data system has arrived and has been found to be more versatile and efficient than the original system. Slight changes in the format of some of the data will be apparent as a result of this new acquisition.

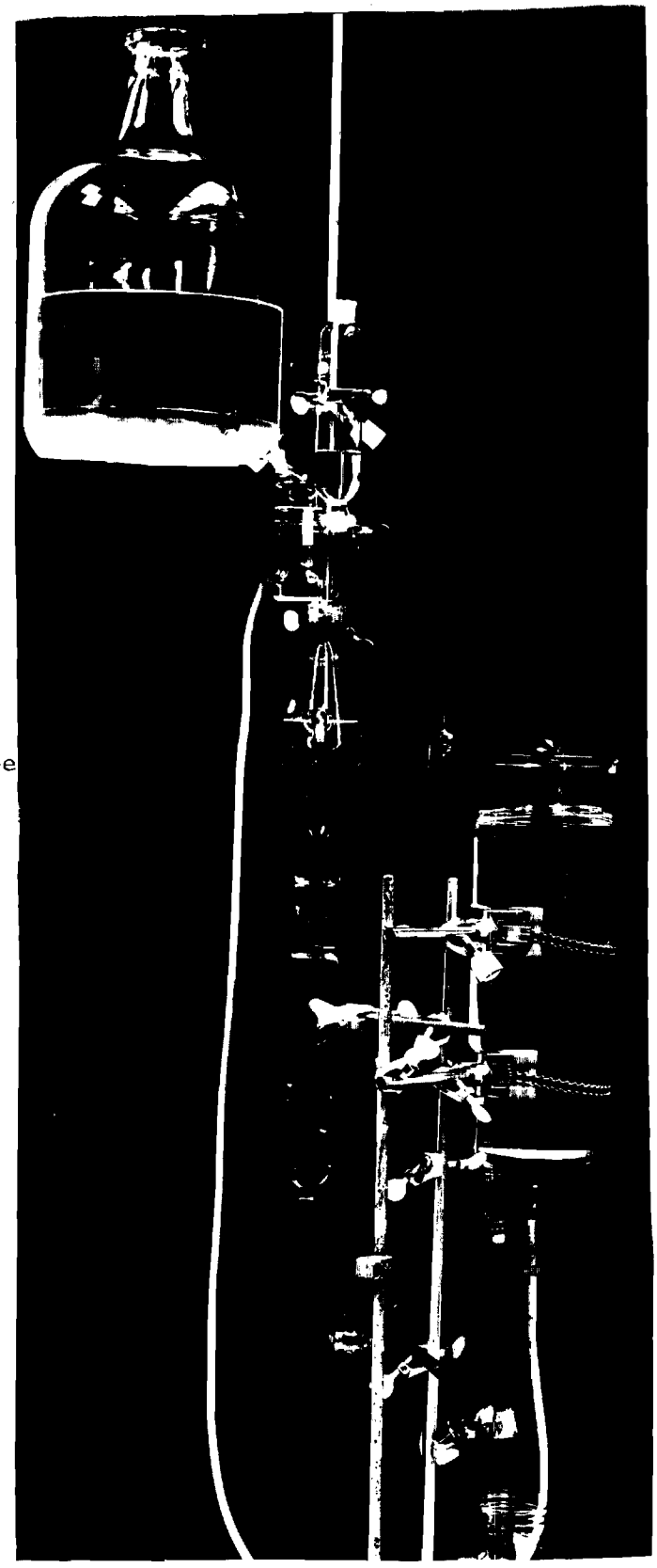


Figure 1. Photograph of Mini-plant for Study of Water Disinfection and Purification

GAS CHROMATOGRAPHIC STUDIES

The gas chromatographic studies completed during December were done with the GC/MS system. The GC conditions used for examination of samples from ozonization of methylated aquatic humic material were:

column: 25m X 0.8mm o.d. glass capillary

stationary phase: SE-30

carrier gas: He

split flow: 46 ml/min.

sweep flow: 57 ml/min.

temperature program: 140° to 180° at 10°/min;
180° to 220° at 1°/min. with a hold at 225° for 10 min.

Injector: 250° C Transfer Line: 265°

Separator: 260° C Ionizer: 250°

The degree of separation achieved under these capillary column conditions is shown in Figure 2 as a total ion chromatogram.

As a measure of progress in our development of capillary column capabilities, a GC/MS analysis of a new work-up of the material which was analyzed at the Finnigan Applications Laboratory in August (see pages 18-22 of the September 2, 1977 Monthly Progress Report) was partially completed. The appropriate total ion chromatogram from that Progress Report is reproduced in this report as Figure 3 for the reviewer's convenience.

The separation shown in Figure 3 was accomplished with a 100 m OV-101 glass capillary column at the Finnigan Applications Laboratory. Figure 4

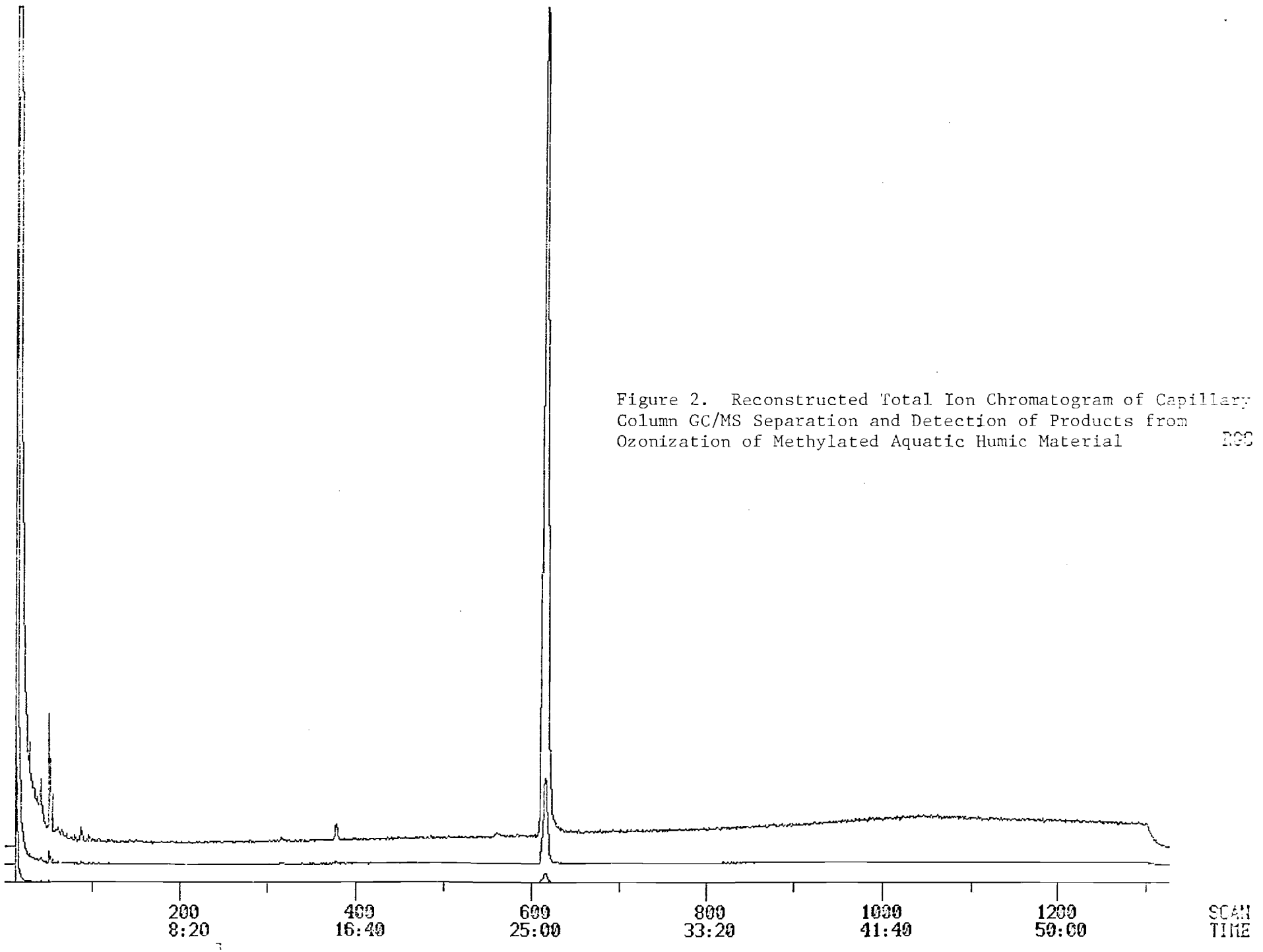
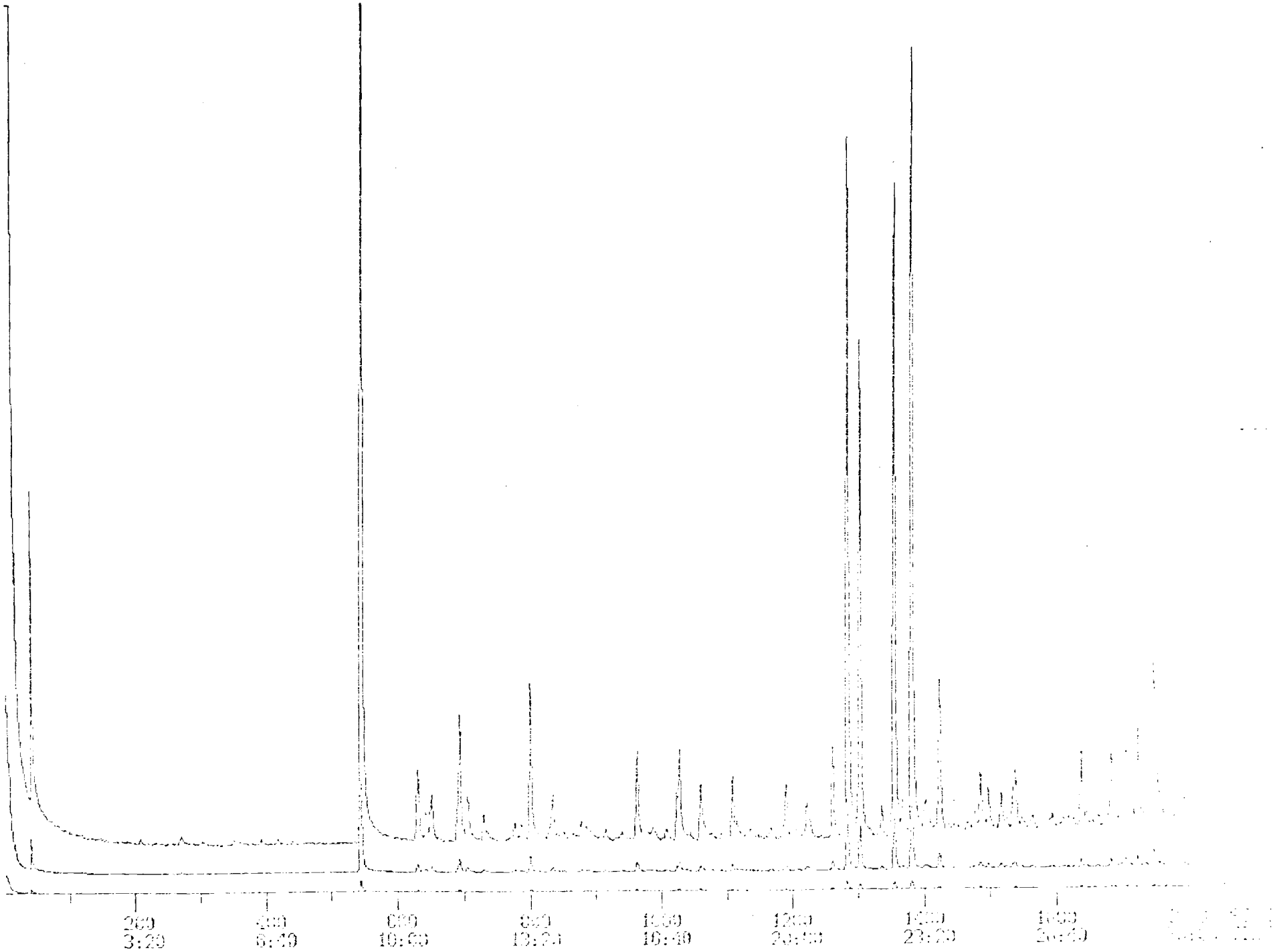


Figure 2. Reconstructed Total Ion Chromatogram of Capillary Column GC/MS Separation and Detection of Products from Ozonization of Methylated Aquatic Humic Material

Figure 3 Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II.
Scans 1-1800 (not enhanced)



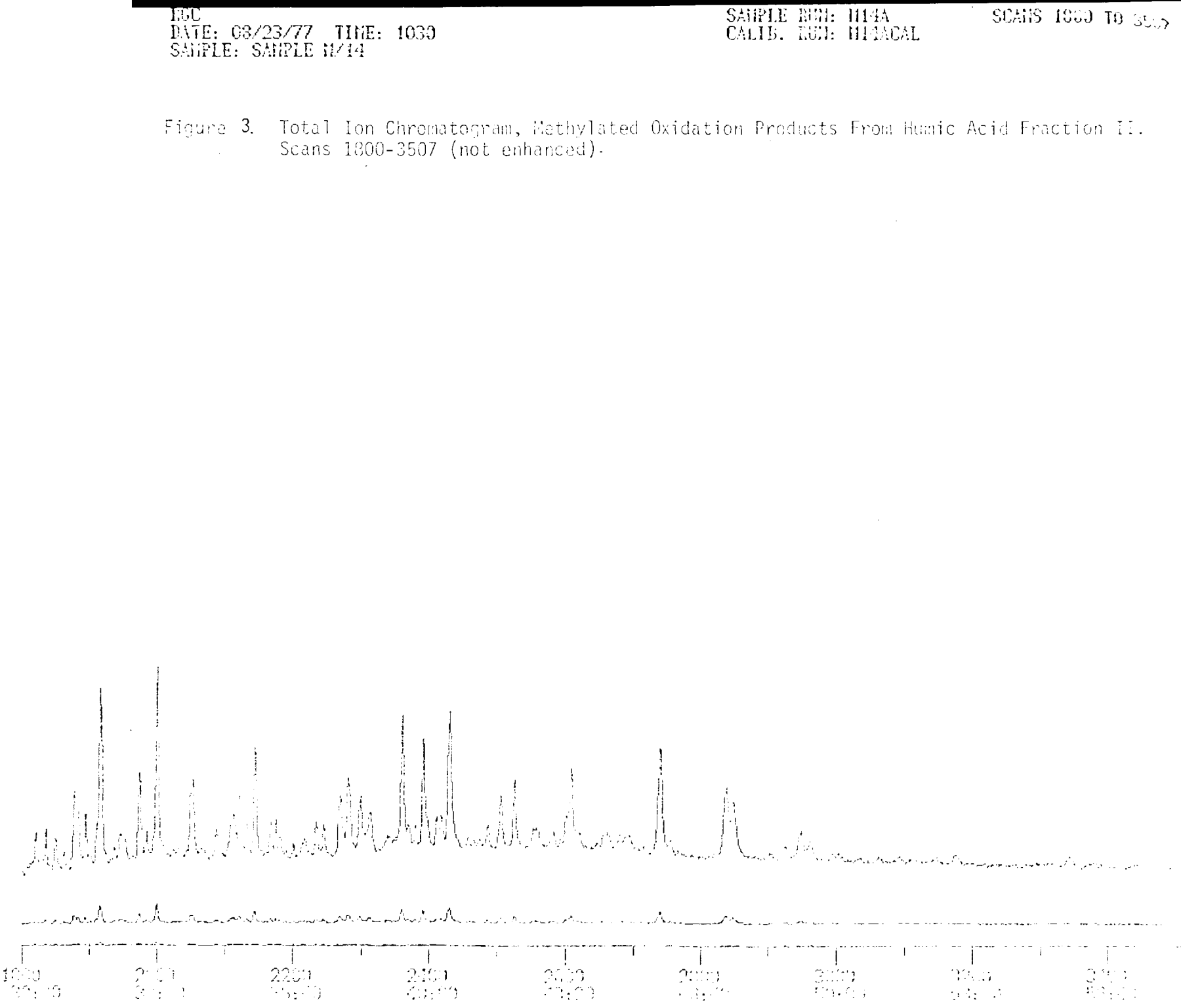
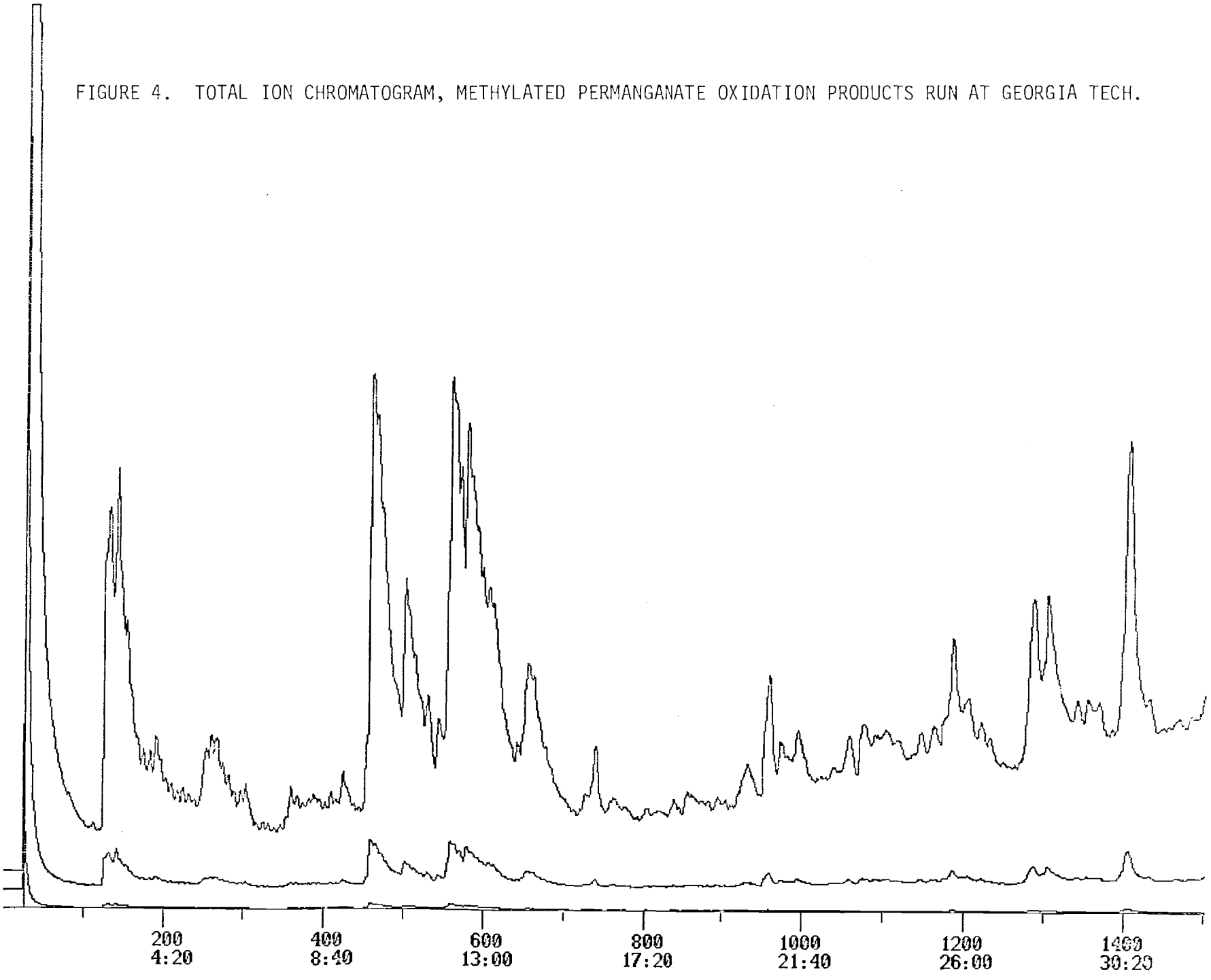


Figure 3. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Fraction II. Scans 1000-3507 (not enhanced).

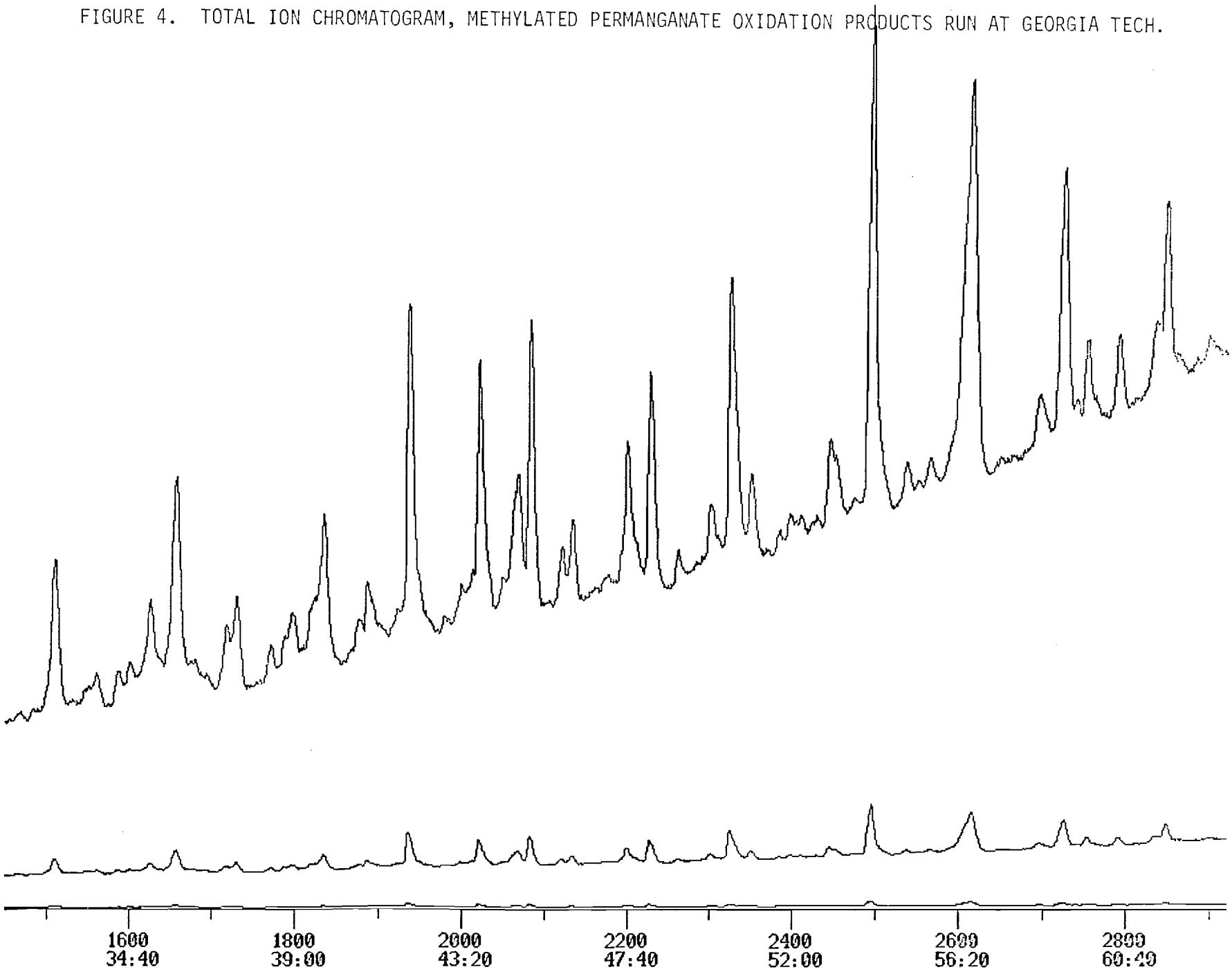
SAMPLE: M/14 RERUN

FIGURE 4. TOTAL ION CHROMATOGRAM, METHYLATED PERMANGANATE OXIDATION PRODUCTS RUN AT GEORGIA TECH.



SAMPLE: M/14 RERUN

FIGURE 4. TOTAL ION CHROMATOGRAM, METHYLATED PERMANGANATE OXIDATION PRODUCTS RUN AT GEORGIA TECH.



shows a preliminary separation achieved on a 25 m SE-30 column at Georgia Tech. (Please note that the two parts of Figure 4 overlap by 50 scans.)

The conditions used for this separation were the following:

Column: 25m x 0.8 mm glass

Stationary Phase: SE-30

Carrier Gas: He

Split Flow: 38 ml/min.

Sweep Flow: 115 ml/min

Temperature Program: 100° to 200° at 1.5°/min.

(run terminated at approximately 165°)

Injector: 250° Transfer Line: 258°

Separator: 250° Ionizer: 250°

The sweep flow was set at an unusually high level because a new septum had just been installed.

The run terminated itself when the data storage disk became full, and is therefore incomplete and will have to be repeated. In comparing the two chromotograms, it would be incorrect to conclude that we have a long way to go in achieving state-of-the-art level of competence in capillary GC/MS techniques. The poorer resolution, particularly in the earlier stages of the chromatogram, can be attributed mostly to differences in the viscosities of the SE-30 used by Georgia Tech and the OV-101 used by Finnigan. The shorter column length available to Georgia Tech is another factor accounting for the somewhat poorer resolution shown in Figure 4. We believe that we are now ready to extend our capabilities by investing in longer columns and a broader range of stationary phases. Work is continuing with the Capillary - EC system.

We are pleased to mention that we have been invited to present a paper entitled "The Use of a Novel Gas Chromatographic Detection System for the Analysis of Trace Halo-Organics" at the National Bureau of Standards 9th Materials Research Symposium. A copy of this presentation will be made available to the sponsor for approval prior to release.

ISOLATION OF AQUATIC HUMIC MATTER

A second trip was made to southeastern Georgia in early December in order to collect additional water samples from the Satilla River flood plain. The point of collection and the method of collection have already been described in the November 4, 1977 Monthly Progress Report, page 9. The water flow in the tributary sampled was two to three times greater than during the October, 1977 sampling period.

CHARACTERIZATION OF HUMIC MATERIALS

An acidic aqueous solution of aquatic humics was treated under reducing conditions in order to estimate the relative contribution of aldehydic, ketonic and quinonoid carbonyl groups to the overall absorption in this region of the infrared spectra of aquatic humic fractions.

A solution of 0.2 g humic material in 15 ml of high-purity water was treated with 0.12 g of triethylamine borane under a nitrogen atmosphere for 5 minutes at 65° and allowed to stand an additional 2 hours at 25°. The resulting solution was treated with excess sodium bicarbonate, and desalted with acidic cationic exchange resin.

A comparison of the infrared spectra prior to treatment (Figure 5) with the spectra after treatment (Figure 6) indicates a reduced absorption or sharpening of the hump between the carbonyl peak at 1725 cm^{-1} (aromatic ester) and the carbon-carbon double bond peak at 1600 - 1630 cm^{-1}. Since carbonyl groups of the type susceptible to attack by the reagents employed in this reaction all lie between 1675 cm^{-1} and 1715 cm^{-1}, these subtle results can be regarded as evidence that groups of this type (aldehyde*, ketone* and quinone are present to at least some extent in natural aquatic humic materials.

* These are most likely to be predominately aromatic in nature and the range would therefore be compressed to 1675 cm^{-1} - 1700 cm^{-1}.

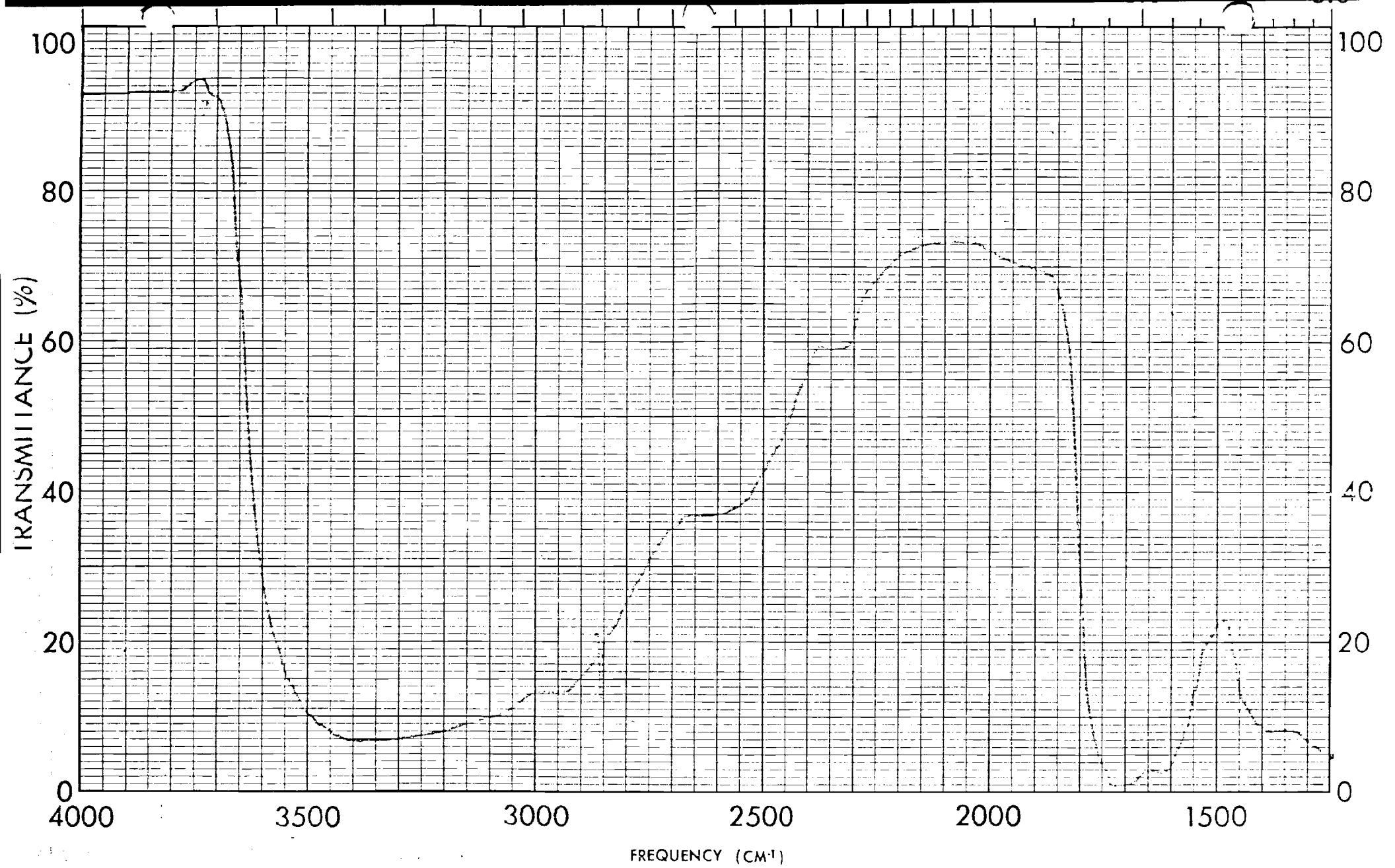


Figure 5. Infrared Spectrum of Desalted Aquatic Humics (KBr Pellet)

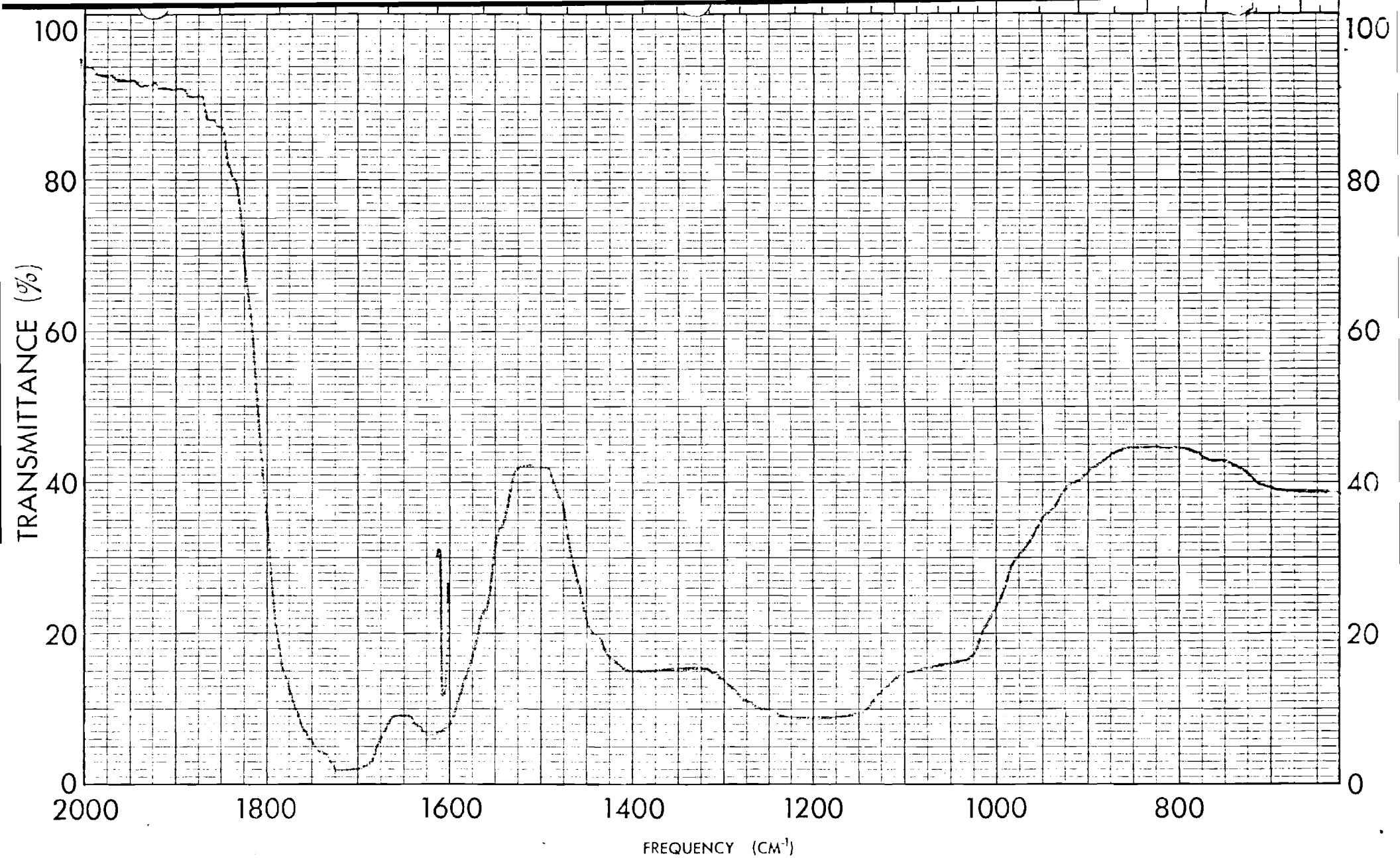


Figure 5. Infrared Spectrum of Desalted Aquatic Humics (KBr Pellet)

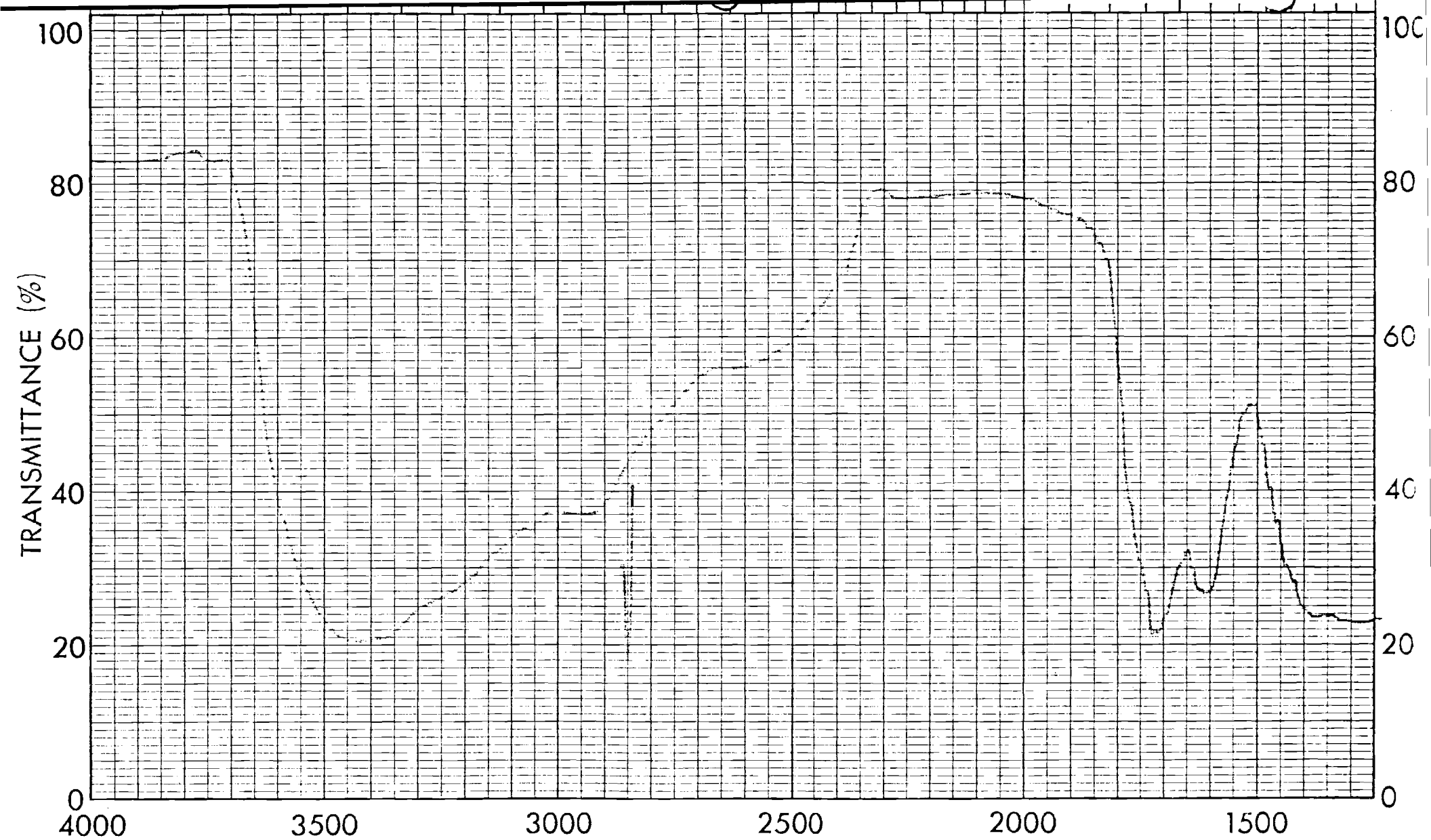


Figure 6. Infrared Spectrun of Reduced Aquatic Humics (KBr Pellet)

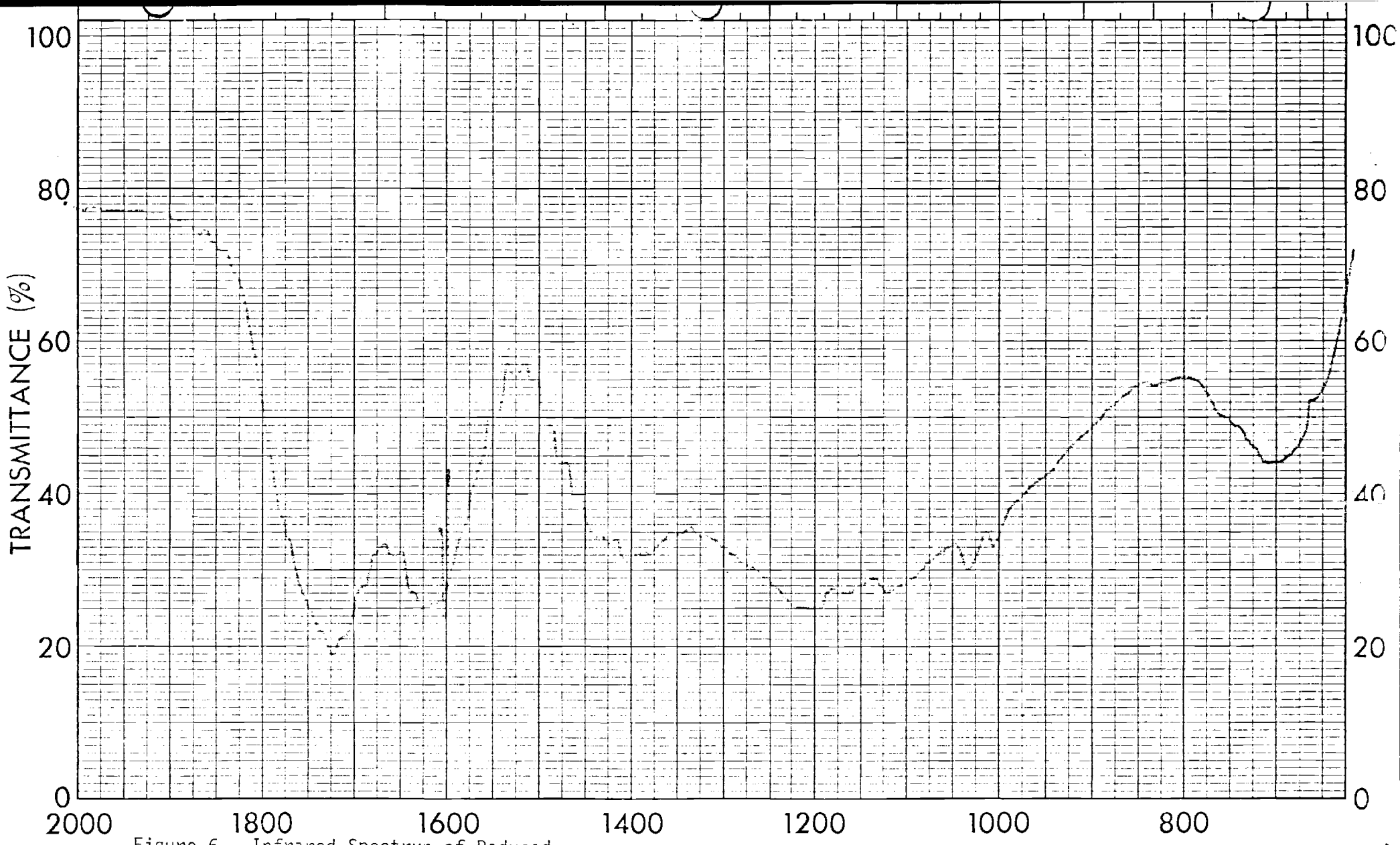


Figure 6. Infrared Spectrum of Reduced Aquatic Humics (KBr Pellet)

The reduction product was further methylated with diazomethane to give a gummy material which was only partially soluble in chloroform. It has not been possible, as yet, to form a satisfactory KBr pellet with the gummy material in order to obtain a meaningful infrared spectrum.

The reduced and methylated material from aquatic humics will be used for further degradation studies. A reaction of this material or fractions thereof with periodic acid will assist in locating adjacent hydroxy groups.

OXIDATION OF METHYLATED AQUATIC HUMICS

A second component of the mixture resulting from the ozonization of methylated aquatic humic matter (described on pp. 7-12 of the December 5, 1977, monthly report) has been identified as trimethyl citrate. The reconstructed total ion chromatogram for the separation of the mixture in the region where the trimethyl citrate appears is shown in Figure 7. A comparison of the mass spectrum of the component in question with the reference spectra stored in our GC/MS data system resulted in a good fit with the spectrum of trimethyl citrate (see Figure 8).

A 1 g portion of aquatic humic material was treated four times with excess diazomethane in order to accomplish complete methylation. The infrared spectrum, shown in Figure 9, exhibits the expected differences from the infrared spectrum of the starting material. The proton magnetic resonance spectrum (Figure 10) shows a strong signal due to methyl protons (δ 3.4-4.0) as well as the presence of protons on aromatic rings (δ 7.35) and aliphatic chains (δ 0.8-1.3).

The aforementioned methylated aquatic humic material was heated under

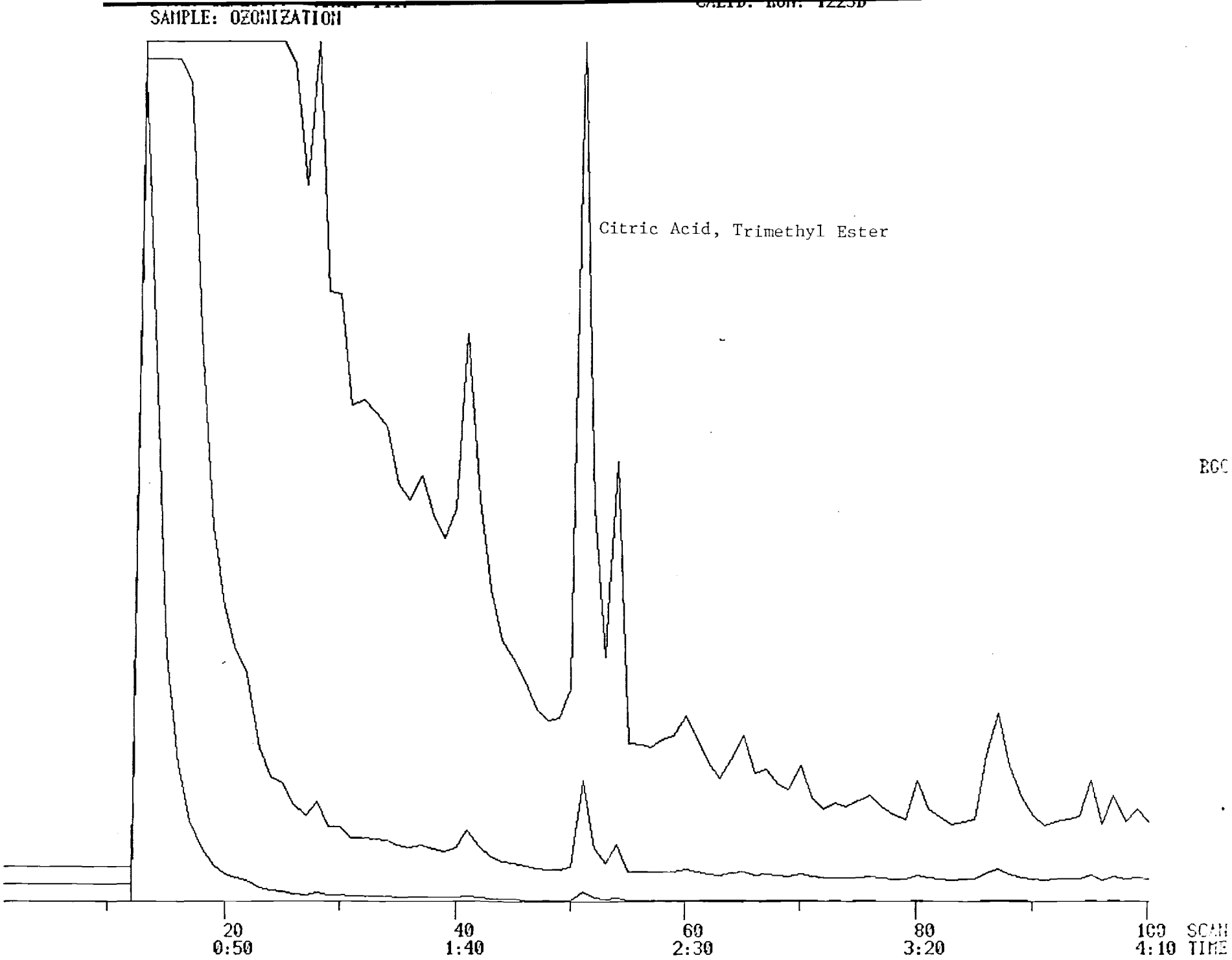


Figure 7. Reconstructed Total Ion Chromatogram of Ozonation Products from Methylated Aquatic Humics

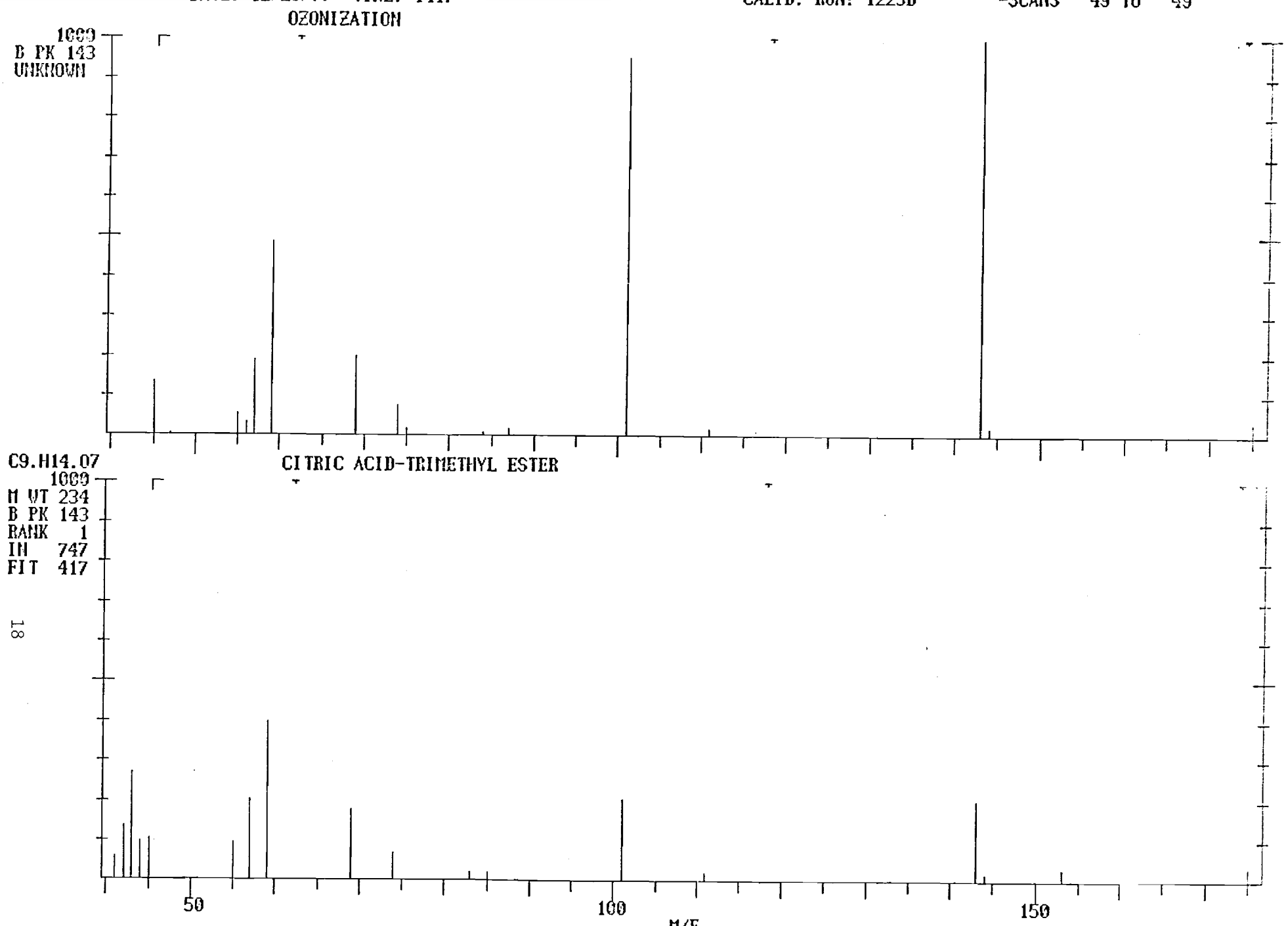


Figure 8. Fragmentation Patterns for Compound Isolated from Ozonized Methylated Aquatic Humics and Reference Library

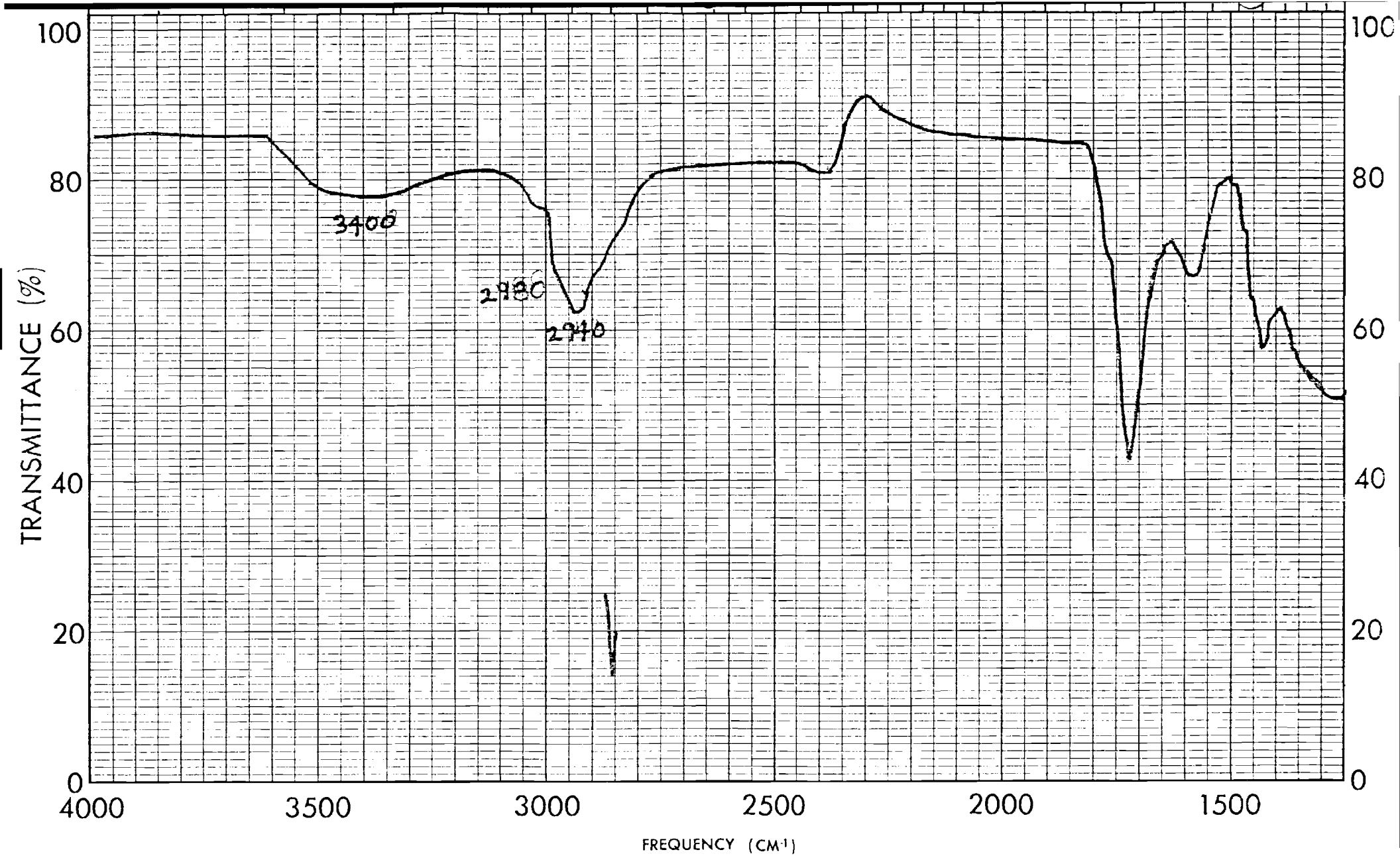


Figure 9. Infrared Spectrum of a Methylated Aquatic Humic Fraction

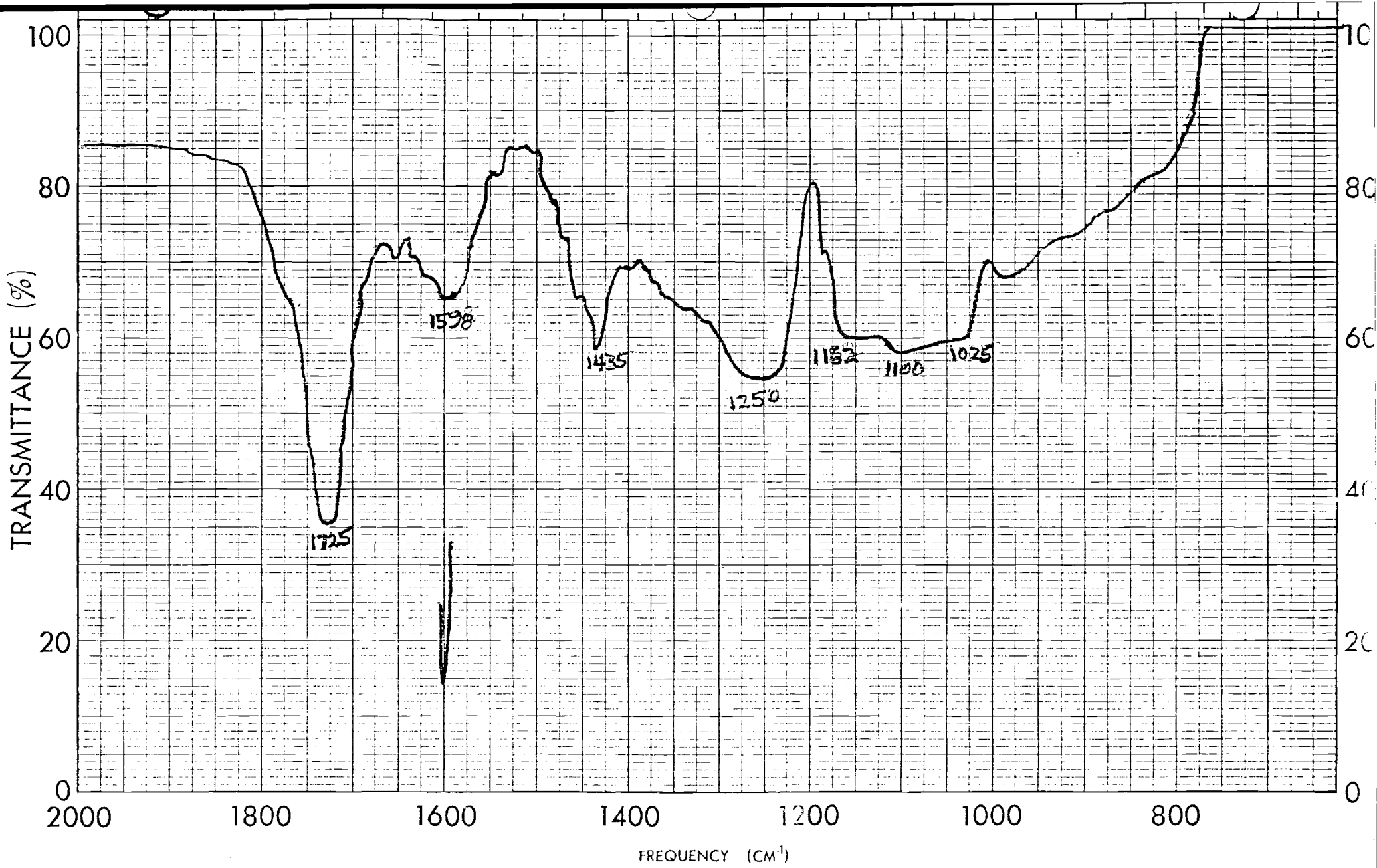


Figure 9. Infrared Spectrum of a Methylated Aquatic Humic Fraction

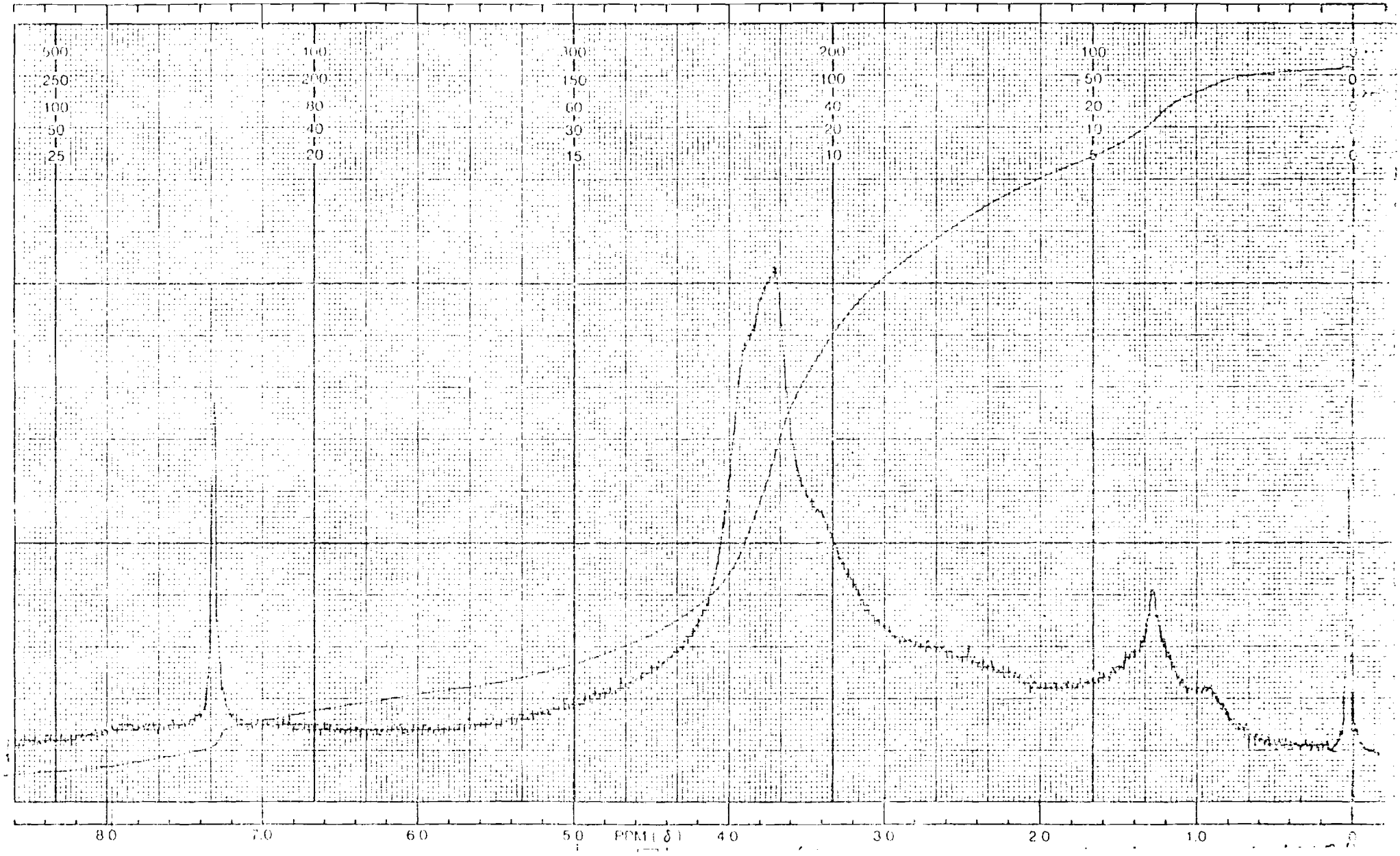


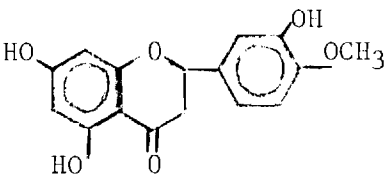
Figure 10. Proton Magnetic Resonance Spectrum of Methylated Aquatic Humic Fraction

reflux with 4% aqueous potassium permanganate for 15 hr. The resulting solution was filtered, treated with more solid potassium permanganate, and heated under reflux for 24 hr. The excess oxidant was then destroyed by the addition of high purity methanol. This reaction mixture containing the new organic acid functions generated by the oxidation reaction was filtered, freeze-dried, dissolved in 30 ml of high purity water, and treated with the minimum amount of acid needed to protonate the free carboxylic acid functions prior to extraction with high purity ethyl acetate. Upon evaporation of solvent a material with an odor characteristic of low molecular weight aliphatic acids was obtained. In this case the relative yield of acidic material was higher than had been obtained in earlier oxidations. After further methylation the composition of this oxidation product mixture will be investigated using the GC/MS system.

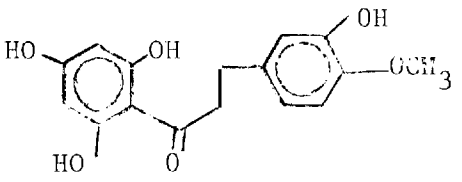
FUTURE EXPERIMENTS

Activity during the next reporting period will emphasize liquid chromatographic methods so that non-volatile constituents may be more thoroughly characterized from both the factorial series and the mini-pilot experiments. In view of the unsatisfactory situation which resulted from use of badly contaminated coniferyl alcohol, efforts are being directed toward the use of hesperitin as a model compound, providing a satisfactory degree of purity can be achieved. In order to avoid further loss of time we shall simultaneously seek to obtain samples of the dihydrochalcone sweeteners. Since these materials are being tested for commercial food use, highly purified authentic standards should be readily available. Hydrolysis or other means of sugar removal would provide the desired 1,3-dihydroxy aromatic structural feature which is believed to be involved in the production of trihalomethanes upon

treatment with chlorine. In addition, other reactive sites are also present in both molecules which could serve to generate a wide variety of oxidation and/or halogenation products. Since both hesperitin and the dihydrochalcones are natural products of some complexity, they incorporate many of the structural features believed to be present in natural aquatic humic substances and would therefore be good choices for this important work. The structures of these compounds are presented below.



hesperetin



neohesperetin dihydrochalcone

The mini-pilot experiments will proceed according to the following general plan. The reservoir will be filled with high purity water and agitated prior to removal of the water blank. Blanks will also be taken of each of the chemicals used in the water treatment process including the model compound itself. The model compound will be added to the reservoir and thoroughly mixed prior to initiation of flow. As soon as the constant head device fills and the test solution enters the mixing chamber, the addition of chemicals will begin. Roasted alum will serve as the coagulant. A solution prepared from roasted sodium carbonate and high purity hydrochloric acid will serve as the buffer (pH 7.2). The chlorine will be generated by acidification of a solution of hypochlorite which has been subjected to a preliminary nitrogen sweep to remove any volatile materials which might otherwise be swept into the chlorine solution which is to be added to the test solution in the mixing

chamber. Frequent pH checks in the mixing chamber will be made in order to assure that the pH and residual concentrations are maintained at the desired levels. If desired, hardness can be added by replacing some of the sodium carbonate with roasted calcium oxide. In addition to the samples already mentioned, further samples will be taken at the exit point of the settling chamber, after filtration, after a residence of 4, 8, and 24 hours in the storage reservoir (clear well) , and after final pH and residual adjustment after 0, 4, 8, and 24 hours of standing in order to simulate passage through the distribution system. All samples except the last group will be neutralized to a zero chlorine residual immediately following collection. The last group will be neutralized at the indicated times. Duplicate one liter samples will be taken at each of the indicated points. Blanks will be run in triplicate. We will adjust the chlorine dosage so that a residual of 0.2 mg/l will be maintained in the filter effluent. The final chlorine residual will be adjusted to 1.0 mg/l.

The sponsor is invited to make suggestions and comments regarding this experimental plan.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

February 6, 1978

by

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DISCLAIMER

This report has been reviewed by the Criteria and Standards Division, Office of Water Supply, U.S. Environmental Protection Agency, and is intended for internal circulation only. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

I PERSONNEL

Dr. I. El-Barbary has taken a 10-day leave of absence during the reporting period in order to visit with his family in Egypt. He has now rejoined our staff. No other changes in personnel have taken place during the reporting period.

II. EQUIPMENT

Problems with our LC system appear to have been cured at last so that our planned major efforts in this area can now take place. The GC/MS system was also down for about a week due to simultaneous problems with the memory and a smoky transformer malfunction in the gas chromatograph. An emergency rewiring by our own staff has revived the computer and the GC awaits a part from the manufacturer. As a result of this repair activity, we are learning ever more about how the system works and plan some minor changes in the near future so that the system can process data from our other instruments.

III. HESPERETIN AS A MODEL COMPOUND

A highly-purified quantity of hesperitin has been provided to the laboratory by Dr. Forrest Bayer of the Coca-Cola Company. The molecular structure of this compound is such that one might expect a variety of chemical reactions to take place in the presence of chlorine. Some of the possible reactions are outlined in Figures 1, 2 and 3. The reactions shown, as comprehensive as they are, are not intended to be all-inclusive. We are continuing to expand our list of possible products so that we will have the broadest possible base from which to make future structural assignments.

Figure 1 shows the incorporation of four chlorines into the A ring of hesperitin. The possible incorporation of chlorine into the B ring or the pyrone ring will be ignored in the interest of simplicity. Subsequent attack of one of the α-haloketone functions by hydroxide ion would cause the A ring to open. The resulting carbanion can be protonated to intermediate A or could react directly with chlorine to give intermediate B. Cleavage of A at b would provide the halogenated pyrone carboxylic acid C along with dichloroacetic acid. Cleavage at a seems less likely but might provide methylene chloride and the dicarboxylic acid D. It is interesting to point out that Chian has recently (ES&T, 11, 1177, (1977)) identified methylene chloride as one of the products of chlorination of humic-rich ultrafiltration retentates derived from secondary effluents. Cleavage of intermediate B at a provides a more reasonable route to D. In this case a molecule of chloroform would also be generated. Fission of B at b yields trichloroacetic acid plus C. Up to this point the degradation scheme is analagous to that proposed by Rook (ES&T, 11, 478, (1977)). Attack of hydroxide ion on either A or B to break the molecule apart at C would give the enol acid E plus a molecule of tetrachloroacetone or pentachloroacetone depending on whether A or B was the starting intermediate. Both of these acetone derivatives afford the possibility of further degradation to dichloroacetic acid, trichloroacetic acid, hexachloroacetone and chloroform. Upon loss of a proton, the pyrone ring might open up to yield intermediate F which could become protonated and rearrange to an aldehyde. The resulting di- β-keto acid would be expected to immediately lose carbon dioxide to generate G. Since an aldehyde is likely to remain

Figure 1. Possible Degradation - Chlorination Routes for Hesperitin

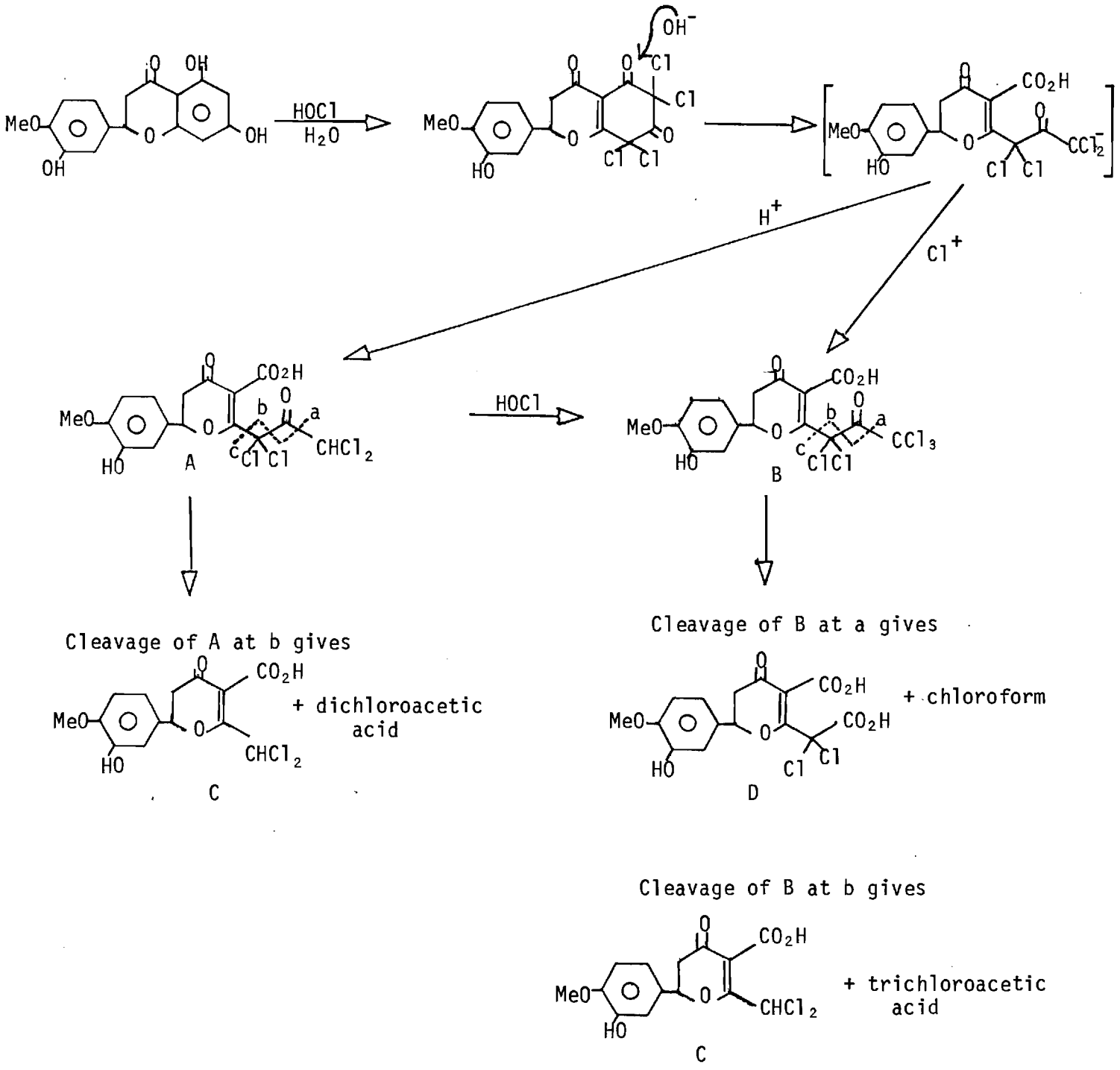
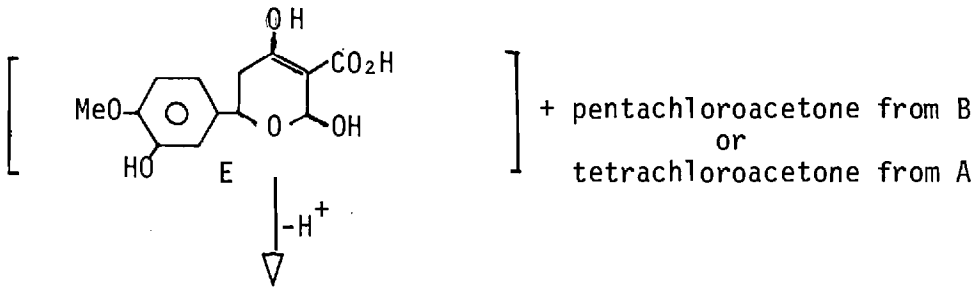
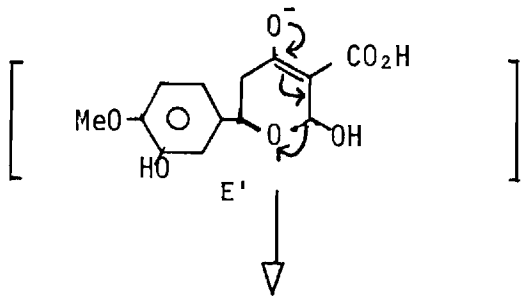


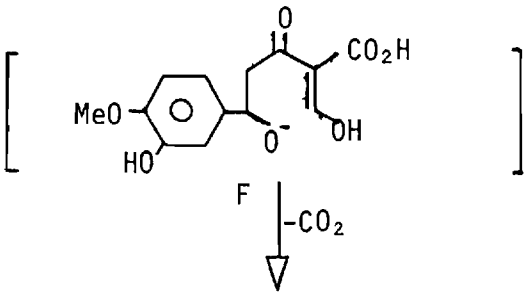
Figure 1. Possible Degradation - Chlorination Routes for Hesperitin
-continued-

Cleavage of A or B at c gives

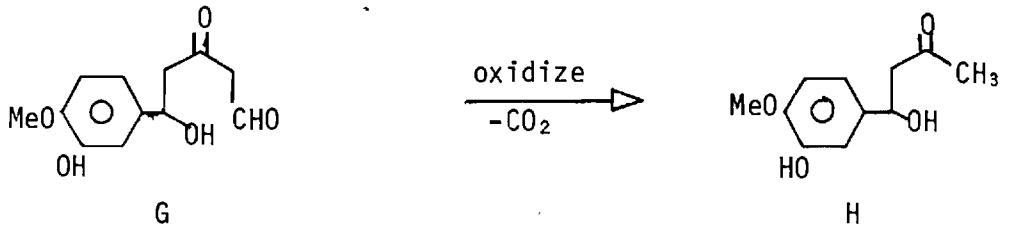


+ pentachloroacetone from B
or
tetrachloroacetone from A





protonate, rearrange to aldehyde



unchanged in an oxidizing medium for very long, the corresponding β-keto acid should form and again lose carbon dioxide to provide the hydroxyketo phenol H.

As might be expected, even compound H is not the end of the list of probable intermediates in this degradation/chlorination sequence. Figure 2 describes the oxidation of the reactive benzylic alcohol function to the corresponding diketone I. Loss of water to form the fully conjugated ketone J is also quite probable. Since I is a reactive diketone halogenation can proceed to intermediate K which, in turn, can cleave at a to form the haloketo acid N plus chloroform. Since N is a β-keto acid, immediate loss of carbon dioxide to the haloketone O would be expected. Compound O might also arise directly from K by scission of the molecule at b. In this case trichloroacetic acid would be the co-product. Fission of K at point c would give rise to the benzoic acid P together with pentachloroacetone. P could also arise from O via conventional haloform reaction. The conversion of the conjugated ketone J to the corresponding trihaloketone L is less likely due to the unfavorable ketone-enol equilibrium. Nevertheless, if it should occur, subsequent conversion to the cinnamic acid M and chloroform would be favorable.

Yet another reaction pathway is possible which seems worth mentioning because it was apparently not considered by Rook in his general reaction scheme. This pathway which is outlined in Figure 3, features the same tetrahalo diketone shown in Figure 1. In this case, however, the keto group between the halogens is the site of attack by the hydroxide ion. Since this site is doubly activated by the flanking pairs of α halogens, it should be the more reactive of the two keto functions. Ring opening at a leads to the dichloroacetic acid anion Q' while opening at

Figure 2. Continued Degradation - Chlorination Routes for Hesperitin

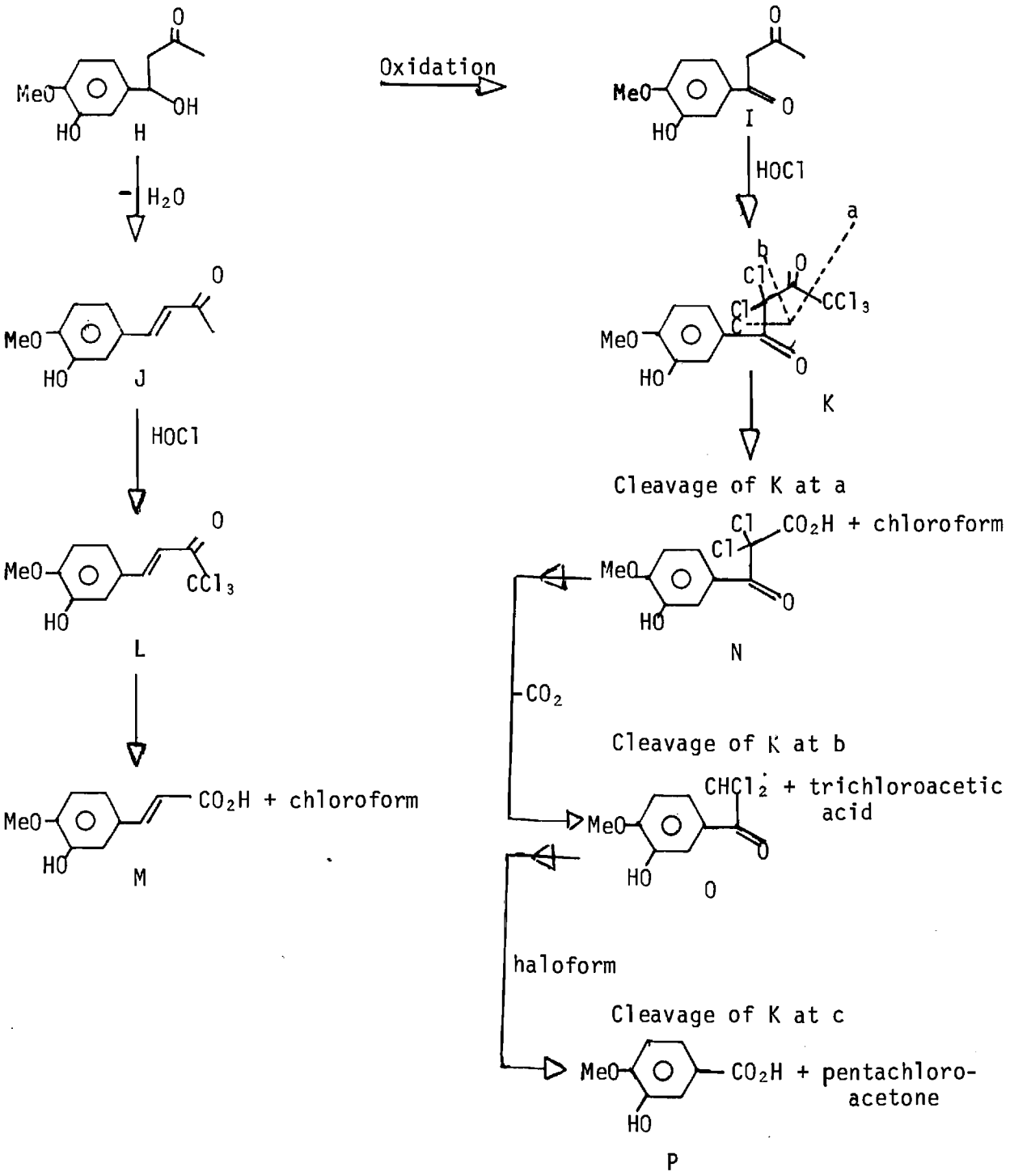
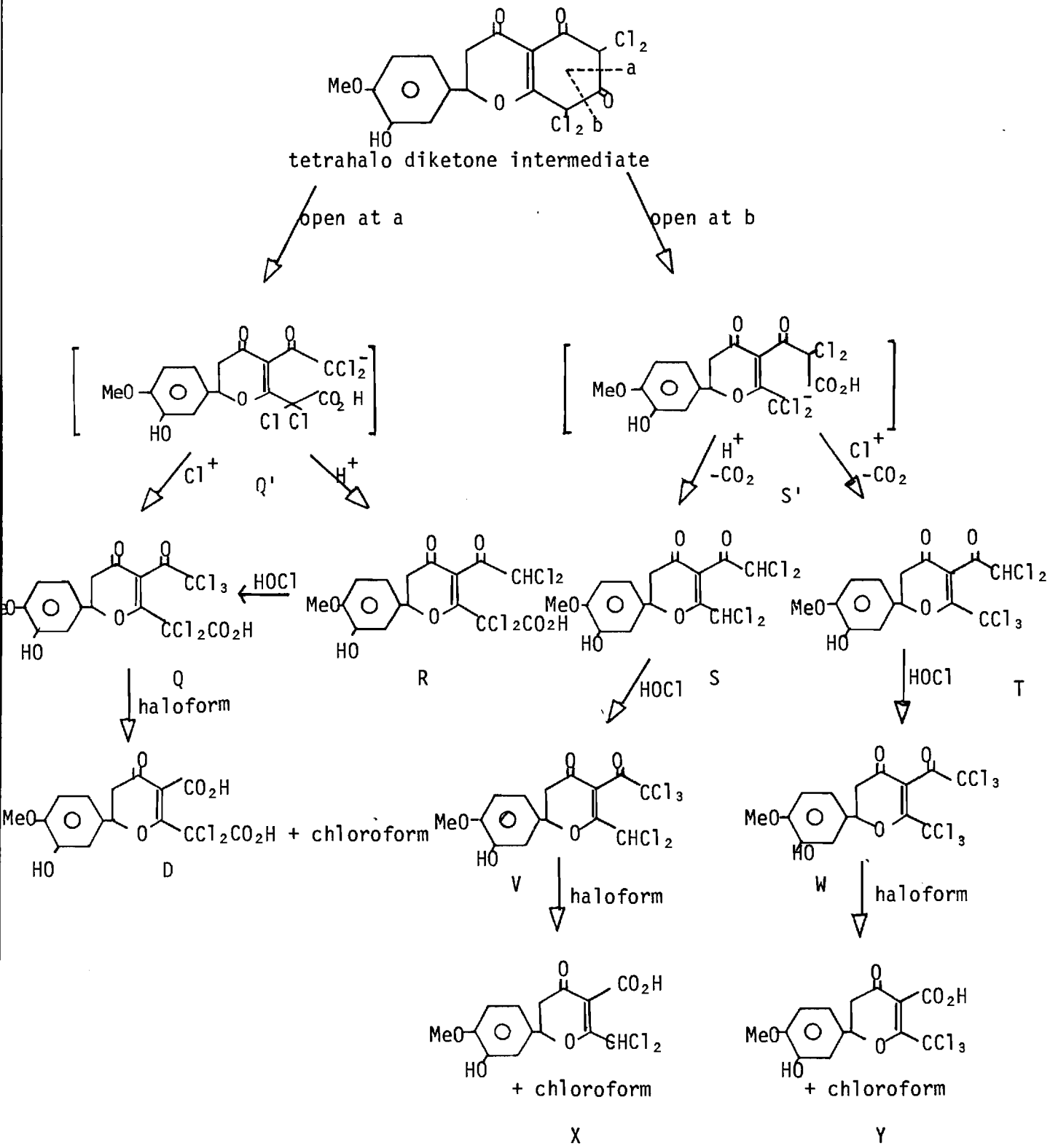


Figure 3. Additional Degradation - Chlorination Routes for Hesperitin



b leads to S'. Reaction of Q' with chlorine provides the pentachloroketo acid Q which can readily undergo a conventional haloform reaction to generate the dicarboxylic acid D plus chloroform. Alternately Q' could become protonated to the tetrachloroketo acid R. This acid could, in turn, react with chlorine to form Q or could add hydroxide ion at position 2 of the dihydropyrone ring giving rise to dichloroacetic acid and leading to a whole series of reactions related to the E, F, G, H sequence of Figure 1. These are not shown.

Intermediate S' has, as a structural feature, a β-keto acid function which should readily lose CO_2—either after protonation to yield S or after reaction with chlorine to yield T. Addition of chlorine to S would be expected to generate the pentachloroketone V which can undergo the haloform reaction to provide X plus chloroform. A similar process starting with T would give the hexachloroketone W and in turn the trihalo acid Y plus chloroform.

Once again, the reaction sequence is not complete, but enough has been presented to be illustrative. Many other reactions are possible:

1 Vinylogous haloform reactions involving the trichloromethyl group of T, W or Y

2 Opening of the dihydropyrone ring by attack at the 2-position

3 Anhydride or lactone formation from D or G and related compounds

4 Internal displacement reactions from D, W, Y etc. leading to the formation of new rings

5 Halogenation of the saturated carbon adjacent to the pyrone function at an early stage in the reaction sequence

6 Halogenation of the B ring of the flavonoid system

7 Oxidation of the A or B rings as an early step

Understanding which of these pathways are favored in a model compound of intermediate complexity such as hesperitin might make it easier to comprehend the infinitely more complex system presented by humic acids.

Furthermore, further hints regarding the structure of humic acids might be gained if more could be learned about favored pathways in the reaction of organic molecules with chlorine under conditions of high dilution. This might permit us to explain the origins of some of Rook and Chian's more unusual halogenated products.

The high-purity hesperitin was found to have a melting point of 222-223^{o}C (uncorrected). Dr. R. Ikan (Natural Products, Academic Press, 1969, p. 12) reports a melting point of 224-226^{o}C. The infrared spectrum (KBr) agrees with what would be expected on the basis of the structure. The UV-VIS spectrum also agrees with published values (λ max = 289 nm, log ε = 4.27)

A 23.4 mg portion of hesperitin was cooled in an ice bath and treated for 10 min. with a solution of diazomethane in ethyl ether until the solid dissolved. The observation of a persistent yellow color indicated the presence of unreacted diazomethane. The resulting solution was warmed to remove excess diazomethane, cooled and diluted with methanol prior to analysis by GC/MS and solids-probe MS.

A comparison of the mass spectra of the reaction products with those of the starting materials showed an unexpected degree of similarity. In fact, the only significant difference between the two sets of spectra was the absence of a peak at m/e 316 in the starting material. This peak represents singly methylated hesperitin.

Two isomers are apparent—propably due to methylation in the A and B rings respectively. Since the 5-hydroxy group is adjacent to the dihydropyrone ring, it is probably strongly influenced both sterically and via hydrogen bonding by the neighboring ketone function. For this reason, a more drastic methylation will be required.

Accordingly, the original reaction mixture was brought to dryness and the residue treated with ten equivalents of diazomethane in ether. The reaction mixture was held at 20-22^{o} for 21.5 hours after which time it was very light yellow. The ethereal solution of methylated hesperitin was extracted with three 5 ml portions of 1N potassium hydroxide to remove any phenolic components still present in the reaction mixture. The resulting colorless ethereal solution was washed with water and dried over anhydrous sodium sulfate. Evaporation of the ether gave 10.1 mg of an oil which was subsequently dissolved in methanol prior to analysis. This work is now in progress.

IV. MINI-PILOT STUDIES

The photograph of the mini-pilot facility shown in the previous monthly report is reproduced this month (see Figure 4) along with a schematic diagram (see Figure 5). A comparison of these two items should help clarify the function of some of the connections which are not clearly defined in the photograph. The reservoir at the upper left corner of both figures is normally charged with a sufficient volume of the test solution plus inorganic buffer to maintain operation for 6-10 hours. The solution leaves the reservoir through a stopcock and enters the constant-head device. The long tygon tube extending downward from the left side of this device is merely a drain and therefore is not a potential source of contamination. The constant-head device itself acts to stabilize the rate of flow of the test solution dropping into the mixing chamber. Chlorine and flocculant are fed into the mixing chamber from a small addition funnel immediately to the right of the constant-head device. Mixing is achieved by means of a magnetic stirrer. The partially treated water

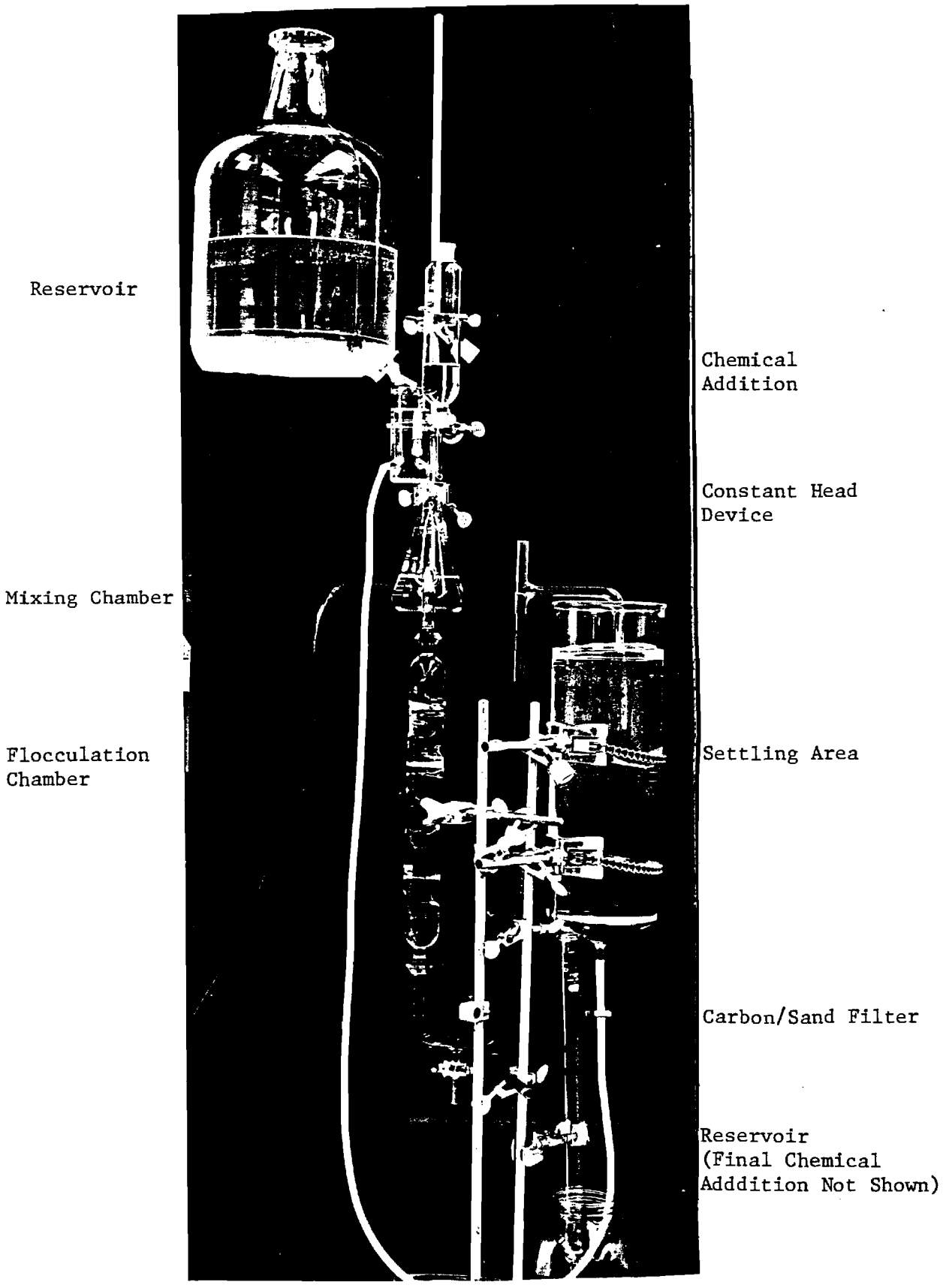
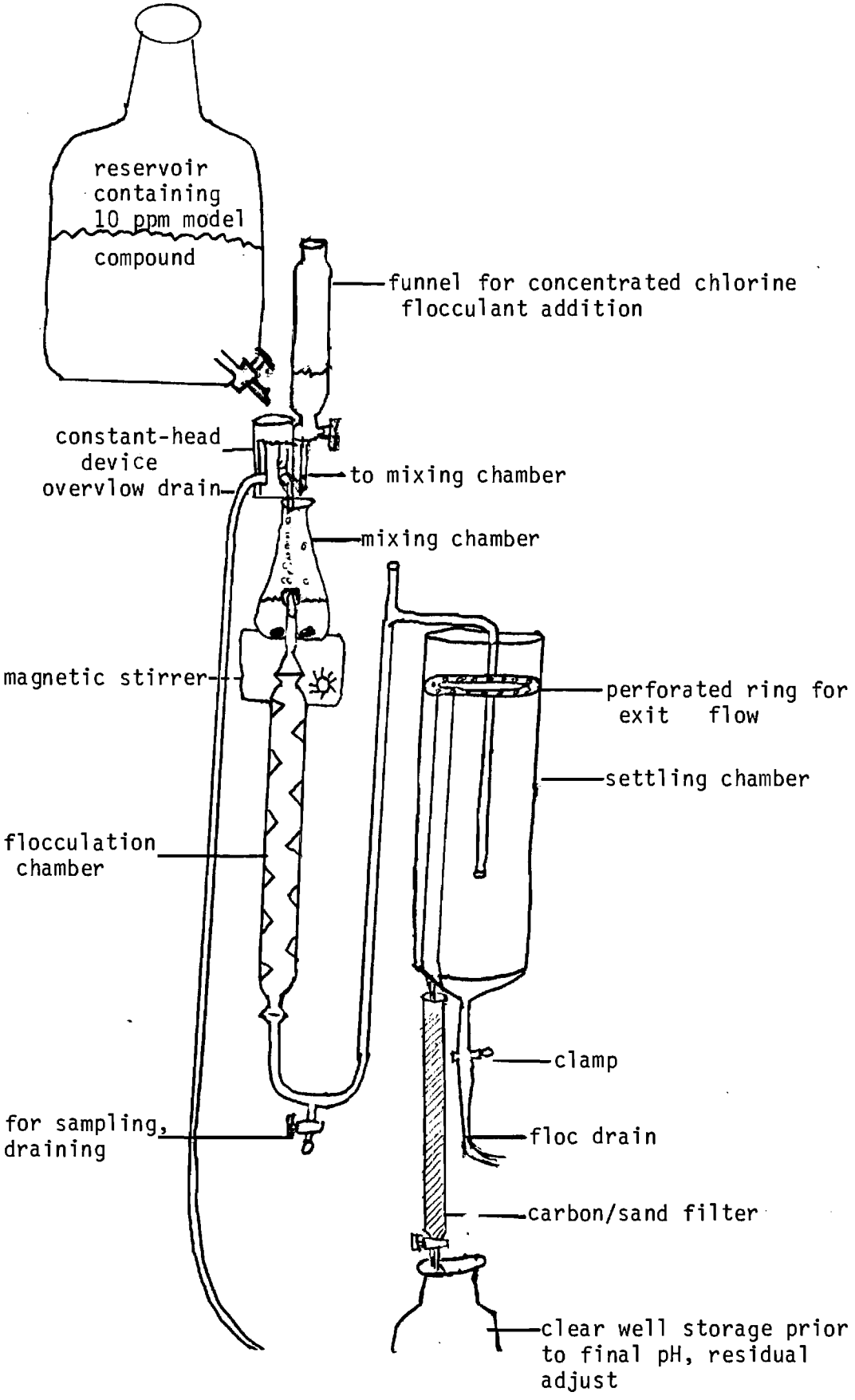


Figure 4. Photograph of Mini-plant for Study of Water Disinfection and Purification

Figure 5. Schematic - Mini-Pilot Facility



leaves the mixing chamber through a glass tube attached part way up the side of the flask (this item is dead center in the photograph and is somewhat difficult to see). The test solution then enters a vertical chamber featuring an array of glass baffles to encourage flocculation. After leaving the chamber in a downward direction, the flow passes a sampling/drainage stopcock on its way to the settling chamber at the right center of the two figures. Entrance of the liquid occurs about 2/3 of the way down the chamber. This settling area features a drain in the bottom center through which floc can be removed. This is equipped with a piece of tygon tubing for convenience. Once again it is not possible for the tubing to contact the circulating water which leaves via a perforated ring at the top of the settling chamber. The resulting exit flow is conveyed to a glass column containing carbon and/or sand through which it flows into a glass "clear well" reservoir for storage prior to final pH and residual adjustment. The surfaces in contact with the test solutions as they flow through the system are entirely composed of Teflon or glass. The system is completely flushed with high-purity water immediately following each run. The modular design will permit changing the sequence of treatment so that for example, carbon filtration could preceed chlorine dosage.

A test run has been conducted with purified resorcinol as a model compound. It was necessary to add 1 g of "roasted" sodium carbonate to the test solution in order to bring the pH to 7.3. Flow from the reservoir was started and as the resorcinol solution entered the mixing chamber, a solution containing 680 mg chlorine (freshly generated from prepurged commercial hypochlorite and dilute sulfuric acid) and aluminum sulfate was added very slowly so as to maintain a constant dosage to the resorcinol solution in the mixing chamber. A 63 minute period was required

to deliver 8.6 liters of the resorcinol solution. The pH of the initial overflow water was 4.5. Since there was no chlorine residual in the effluent from the sand filter, and no floc formation in the flocculation chamber, it was not considered to be cost-effective to work up the product mixture. It was apparent from these results that a better buffer system must be used to maintain the pH of the treated solution at a constant value during the course of the treatment period and to promote floc formation. Other than that, the system operated without any problems. Further work is in progress with resorcinol and with hesperitin. The former experiments are limited in nature and are designed only to establish some common ground between work done at Georgia Tech and work done elsewhere. The experiments with hesperitin are of a more comprehensive nature.

V. RIVER WATER SAMPLING

The water samples collected during December (411 liters) have been completely processed to provide 22.6 g of aquatic humic materials. Another sampling expedition is planned for the near future.

VI. HYPOCHLORITE OXIDATION OF AQUATIC HUMICS

In an effort to more nearly duplicate the reagents employed in conventional water treatment (but not necessarily the reaction conditions), an experiment was undertaken to examine the products resulting from the alkaline oxidation of humic materials with hypochlorite. The oxidant was prepared by adding a hot solution of calcium hypochlorite (15 g) in water (60 ml) to a warm solution of sodium carbonate (8.06 g) and sodium hydroxide (1.428 g) in water (30 ml). An aqueous solution (10 ml) containing 250 mg of humic material was adjusted to pH 12 and added to the mixed, filtered and cooled oxidant solution. The reaction mixture

was allowed to stand at room temperature for 48 hours. The originally dark brown solution faded to a pale yellow color. This solution was then filtered, acidified with hydrochloric acid and extracted continuously with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4) and evaporated to provide a gummy white residue (205 mg). Addition of chloroform gelatinized the residue but did not dissolve it. Treatment with diazomethane did not result in an observable evolution of nitrogen. Further efforts at characterization are in progress. The Beilstein test indicates that this material contains halogens. The fact that ignition leaves no residue suggests that the halogens are organic in nature.

VII. OXIDATION OF AQUATIC HUMICS WITH IODINE IN BASE

A solution of 500 mg humics, 2.5 g sodium hydroxide and 3.7 g iodine in water (25 ml) was heated at 65°C for 30 minutes and left overnight. The dark brown solution was directly extracted with ether. The extracts were washed with water, dried (Na_2SO_4) and evaporated to yield 13.4 mg of yellow hexagonal prisms. The m.p. 122°C (lit. 123°C) and appearance strongly suggest that this material is iodoform. Confirmation by mass spectrometry is in progress. Recalculated as chloroform, the above yield would amount to 0.8% on a weight/weight basis which is in excellent agreement with reported values. The aqueous phase remaining after extraction was cooled and acidified for extraction of acids and phenols. This work is now in progress.

VIII. OXIDATION OF AQUATIC HUMICS WITH ALKALINE PERMANGANATE

A 1.0 g sample of humic material was oxidized in the usual fashion and directly extracted with ethyl acetate to remove neutral and basic products. The aqueous phase was then buffered with a saturated sodium

bicarbonate solution and reextracted with ethyl acetate to remove phenols and other weak acids. The aqueous layer was subsequently acidified with hydrochloric acid and again extracted with ethyl acetate to remove carboxylic acids. Thus far, only the strong acid fraction weighing 225 mg appears to contain material which is amenable to gas chromatographic analysis following treatment with diazomethane. The gas chromatographic pattern is very similar to earlier chromatograms of the total methylated permanaganate oxidation product mixture. This result is not unexpected since organic acids should dominate oxidation product mixtures of this type.

IX. SUMMARY AND PLANS

Liquid chromatographic and capillary GC methods are expected to dominate our product-characterization efforts in the next reporting period. A theoretical study of the possible reaction products resulting from the chlorination of hesperitin strengthens our belief that this compound is a good model for the humic acid system in that a number of reaction pathways each leading to a variety of products are possible.

The mini-pilot facility is in use and work with resorcinol and hesperitin will proceed at a pace which will be determined largely by the speed with which samples can be processed. The same statement can be made regarding the factorial studies. Oxidative studies with chlorine and iodine have provided some interesting results and workup will continue into the next reporting period. The ease with which iodoform can be isolated together with the very good match between our overall yield and literature yields when both are expressed in terms of chloroform suggests that further comparisons may be in order. The characterization of iodoform and other iodoorganics should be much easier than would be

the case for chloroorganics. This factor might permit more rapid progress to be made in establishing reaction pathways. For example, dozens of extractions could be performed simultaneously and results could be obtained gravimetrically. Increased throughput might make up for differences in reactivity between HOCl and HOI—at least until general reaction pathways become more thoroughly defined. The sponsor's comments are welcome.

The use of XAD resins for trapping reaction products will be explored. Current plans call for following the adsorption of organics on the resin with analyis by both x-ray fluorescence and neutron activation methods. In this way a measure of total organic halogen will be provided.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

March 8, 1978

by

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DISCLAIMER

This report has not been reviewed by the Criteria and Standards Division, Office of Water Supply, U. S. Environmental Protection Agency, and is intended for internal circulation only. The contents do not necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

TABLE OF CONTENTS

I. PERSONNEL

No changes in personnel have taken place during this reporting period.

II. EQUIPMENT

The earlier problems associated with the operation of the mass spectrometer, which had been described in recent telephone conversations, have been resolved so that the system is now operating at top efficiency. A new guaranteed-performance capillary column has just arrived. This 60-meter Carbowax 20 M capillary has been tested at 168,000 effective plates and is intended to give us greater flexibility in separating the complex mixtures derived from our humic acid degradation studies, our fractionation experiments and our model compound studies.

The Lupton detector on the gas chromatograph, which was being dismantled for routine cleaning, has been reassembled with a packed chamber and transfer line in an attempt to further minimize dead volume. Hopefully this will reduce recirculation and result in further improvements in performance.

III. ESTIMATION OF CARBOXYL GROUPS IN AQUATIC HUMICS

Two representative samples of aquatic humics from two different sampling periods (M/30 and M-39) were shaken for 24 hours with excess aqueous $Ca(OAc)_2$ solution. The liberated acetic acid was then titrated potentiometrically with standard NaOH solution[1]. A blank sample was subjected to the same treatment.

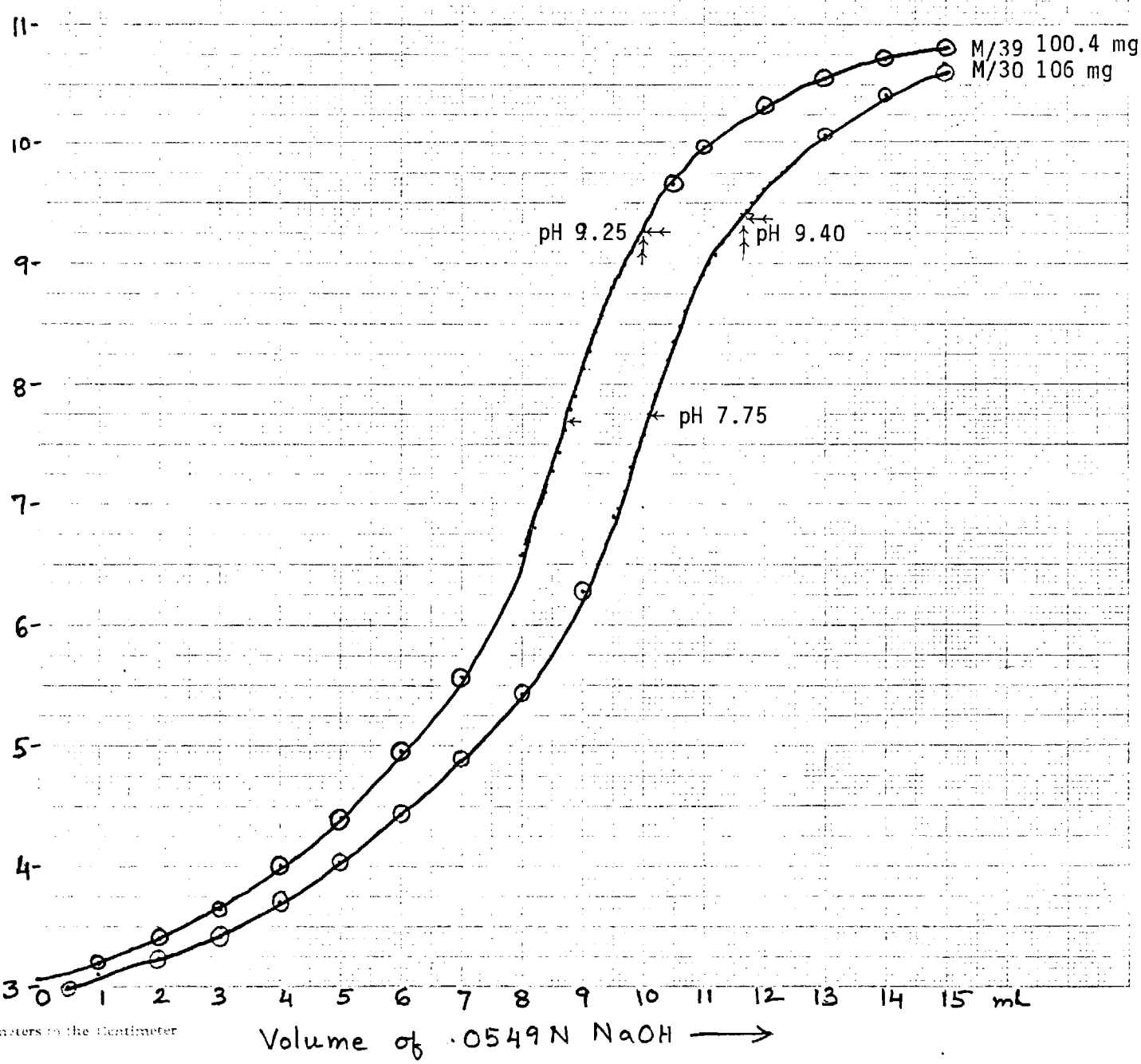
The potentiometric titration curves did not show any sharp inflection (see Figure 1). Previous workers recommended titration up to pH 9.8.[1]

Figure 1. Potentiometric Titration - Humic Acids

⊙ M/30 106 mg

O M/39 100.4 mg

Direct titration of aq. humics in .1N NaCl solution with NaOH.



Our titration curves show that the inflection point occurs before pH 9.8 is reached.

The slope of pH = f(V), where V = volume of alkali added, is so small that it is not possible to determine the equivalence point with accuracy using the above method. Accordingly $\Delta V/\Delta pH$ was plotted against V and the minimum of the curve was taken as the equivalence point[4] (see Figures 2 and 3). It should be pointed out that the blank consumed only 0.2 ml of alkali. The results of these experiments are presented below:

Sample	Carboxyl Groups in meq/g	
M/30	5.55	
	5.59	Avg. 5.57
M/39	5.26	
	5.18	Avg. 5.22

Since aquatic humics may be thought of as polymeric acids, a direct titration with sodium hydroxide in 0.1 N sodium chloride solution was deemed appropriate. A plot of the Gran function $V \times 10^{-pH/n}$ ought to be a straight line and should intersect the second Gran function $K_w(V_0 + V)10^{pH}$ at the equivalence point (n is the slope of the Henderson-Hasselbalch straight portion of a V vs. $-\log \frac{1 - \alpha}{\alpha}$ plot, V is the volume of base added and V_0 is the initial volume of the solution before any base is added). In our case, the Gran functions were not perfectly straight as they exhibited considerable curvature near the end point (see Figure 4). Therefore, the linear portions away from the equivalence point were extrapolated to an intersection which in turn provided the estimate of the end point. The results of these experiments are presented below:

EQUIVALENCE POINTS
HUMIC ACID TITRATIONS

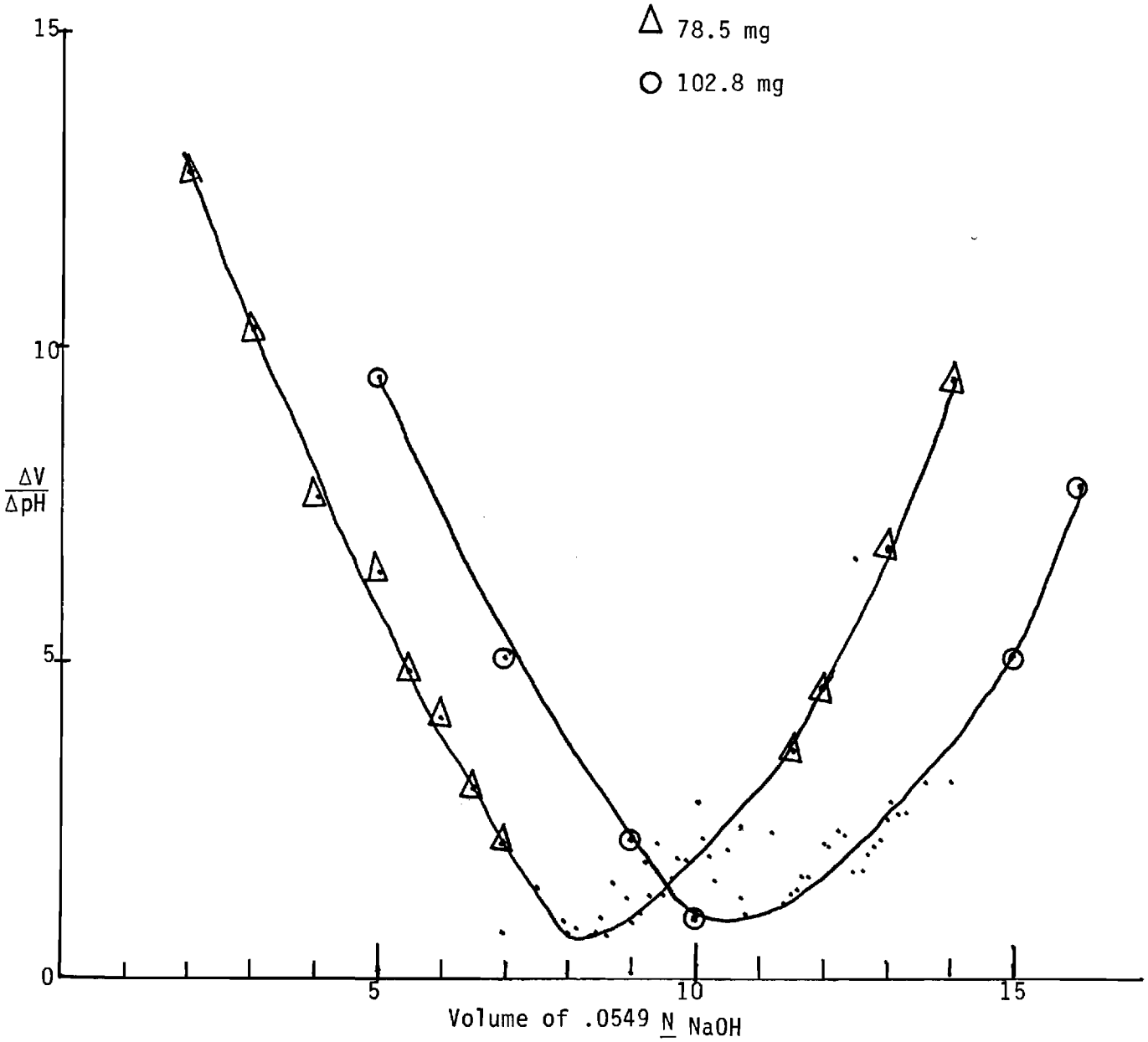


Figure 2. Sample M/30.

EQUIVALENCE POINTS
HUMIC ACID TITRATIONS

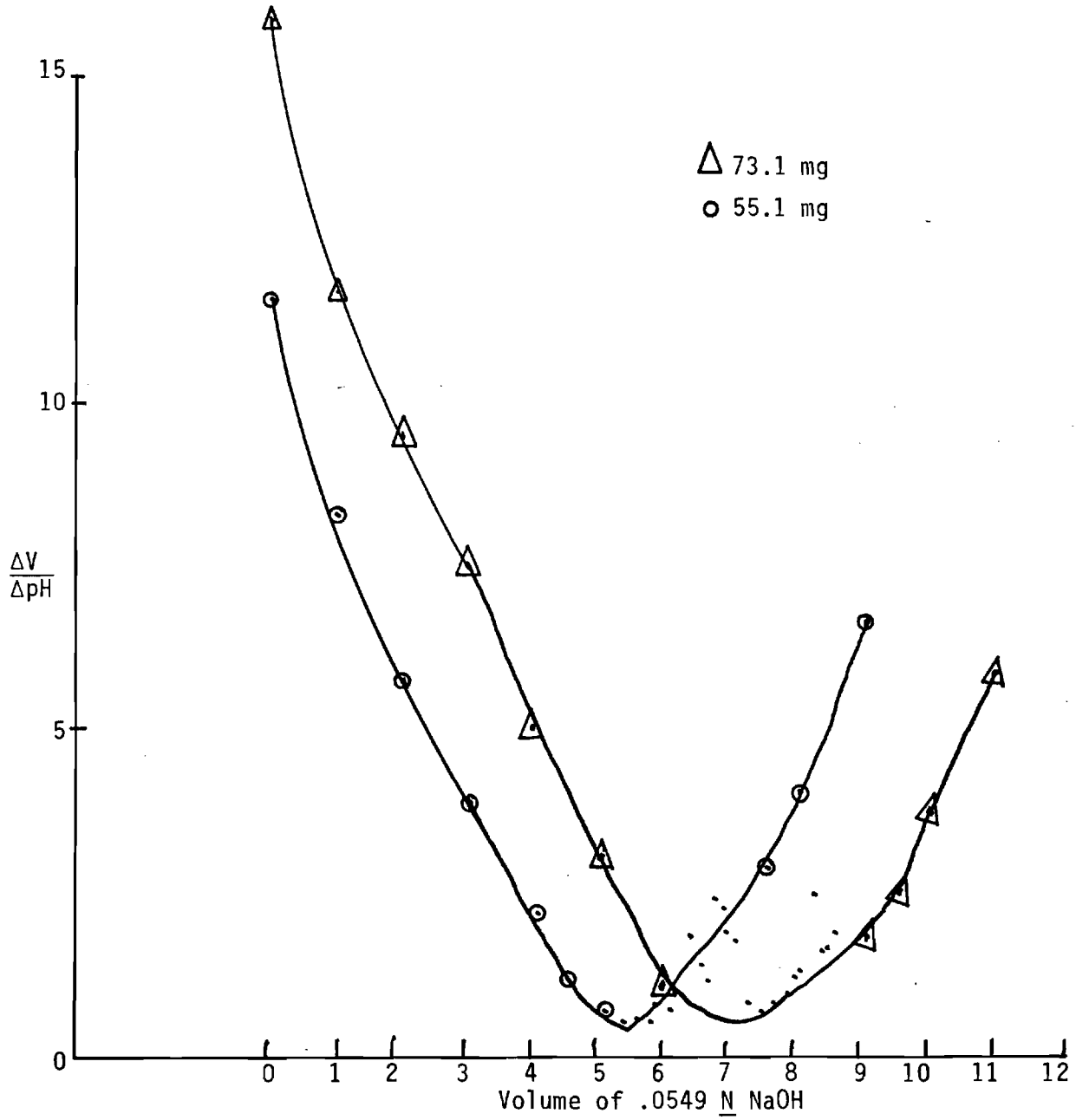


Figure 3. Sample M/39.

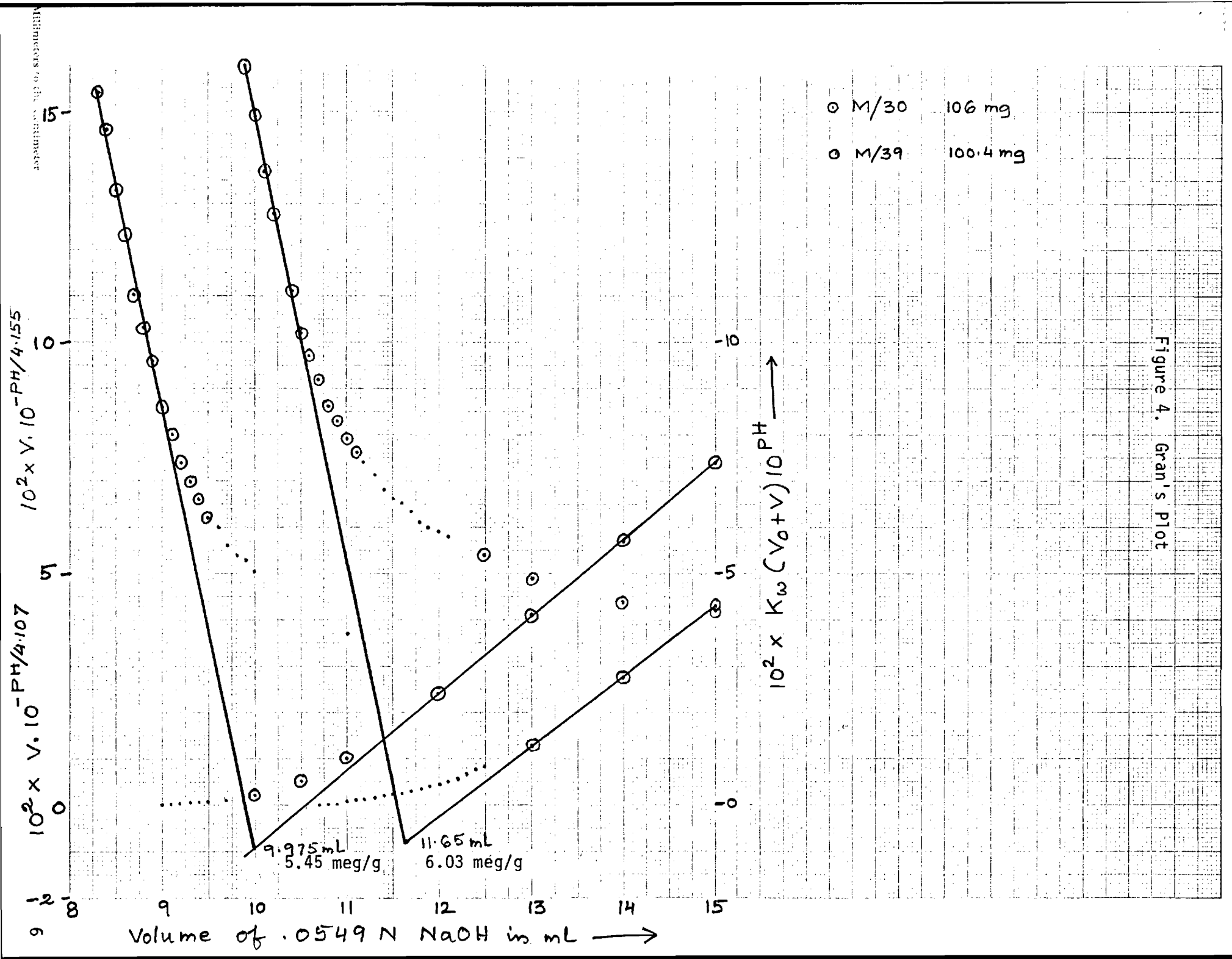


Figure 4. Gran's Plot

Sample	Carboxyl Groups in meg/g Direct Titration	Acetate Method
M/30	6.03	5.57
M/39	5.45	5.22

IV. ESTIMATION OF TOTAL ACIDITY OF AQUATIC HUMICS

In this case, subsamples of the previously described humic acid samples (M/30 and M-39) were allowed to react with an excess of pre-standardized barium hydroxide solution for 24 hours. The unreacted barium hydroxide was then back-titrated with standard acid.[5] A blank was run under the same conditions and similarly titrated. All operations were carried out in a nitrogen atmosphere in order to avoid confusing the results with competing oxidative reactions. This work is still in progress and the duplicate results to those presented below are not yet available.

Sample	Total Acidity in meq/g
M/30	11.1
M/39	10.9

If one subtracts the carboxylate acidity from the total acidity, one obtains an estimate of the phenolic acidity. Two comparable pairs of results are obtained depending on whether the direct titration or the acetate results are used. They are presented below.

Sample	Phenolic Acidity in meq/g Direct Titration	Acetate Method
M/30	5.1	5.5
M/39	5.5	5.7

Thus it can be seen that the two kinds of acidity are about equal. In other words, there are about as many free phenolic OH groups in aquatic humic substances as there are free carboxyl groups.

V. SPECTRAL STUDIES

Yet another subsample of the M/30 aquatic humic material isolated from the Satilla River was reduced with trimethylamine-borane complex and methylated with diazomethane. The infrared spectrum of this product could not be recorded without further treatment due to its insolubility in organic solvents and its gummy nature. It was therefore acetylated with acetic anhydride in pyridine. The resulting acetyl derivative is soluble in chloroform and exhibits an infrared spectrum which is free of -OH absorption in the 3100 - 3600 cm^{-1} region (see Figure 5).

Similarly, another subsample was methylated with diazomethane in methanol suspension and then acetylated. The hydroxyl absorption in the IR was found to be greatly reduced upon methylation and did not show up at all following acetylation. These results are shown in Figures 6 and 7.

Unlike the results recently reported in Science,[6] we do not have difficulties in dissolving methylated aquatic humic substances in organic solvents. Therefore, such exhaustive methylation techniques as the use of dimethyl sulfinyl carbanion together with methyl iodide are not required to achieve the goal of solubility in our case. These differences can probably be ascribed to the fact that the Geological Survey group is working with soil humics[7] while we are working with aquatic humics. Thus our methylated product, although it shows some free -OH is soluble in chloroform. Following acetylation, the -OH disappears from the IR while the chloroform solubility remains.

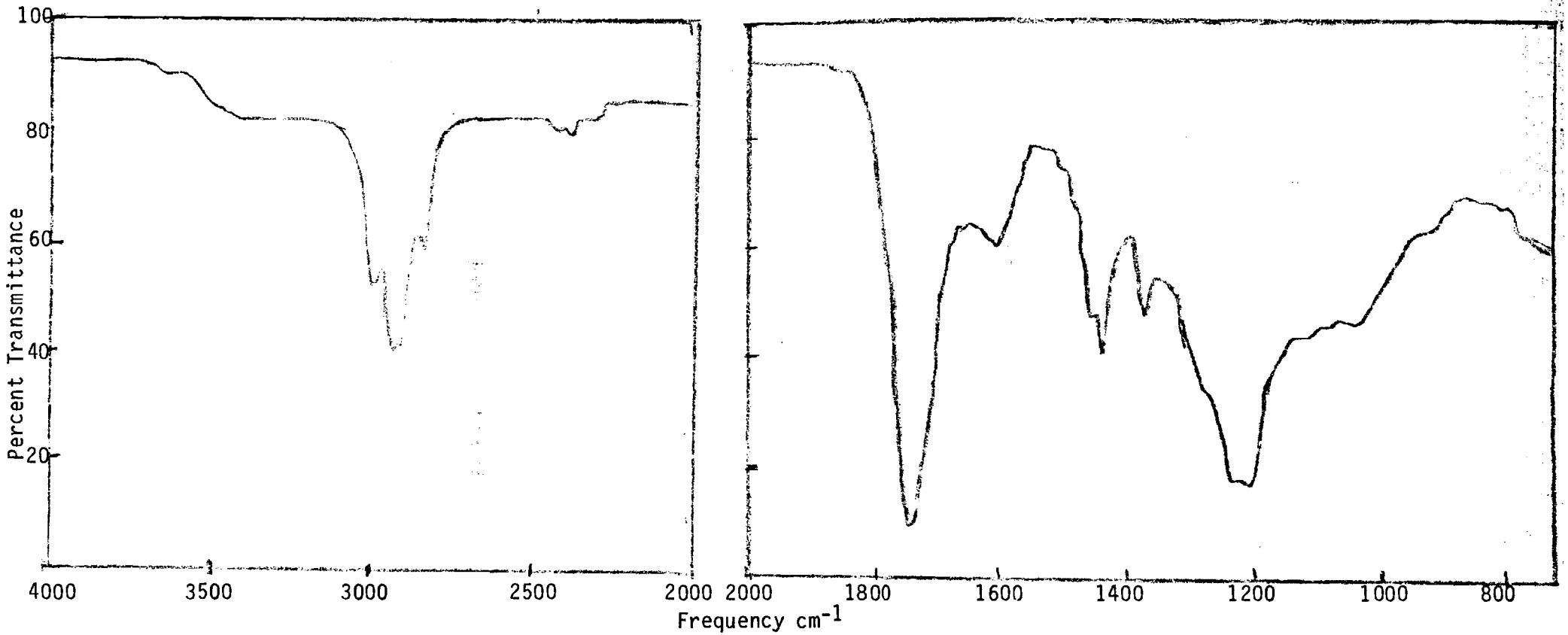


Figure 5. M/35 Reduced, Methylated and Acetylated Aquatic Humic Matter.

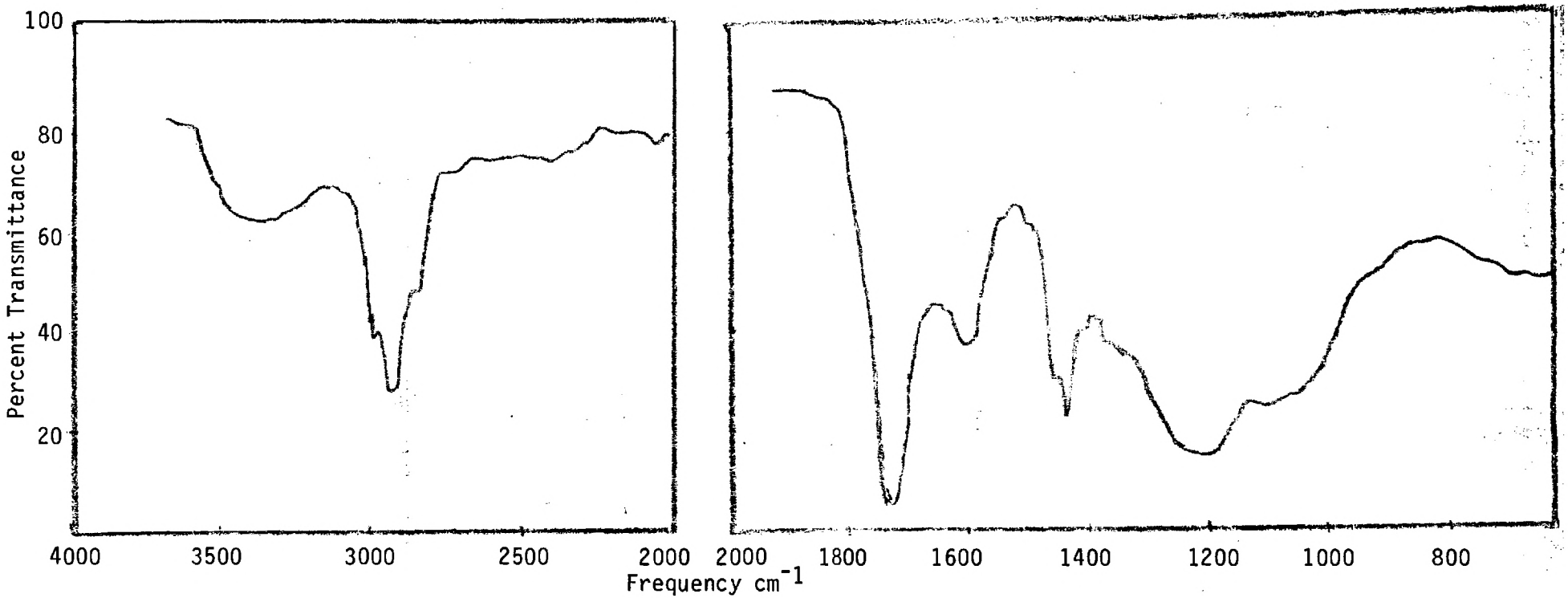


Figure 6. Methylated Aquatic Humic Substances.

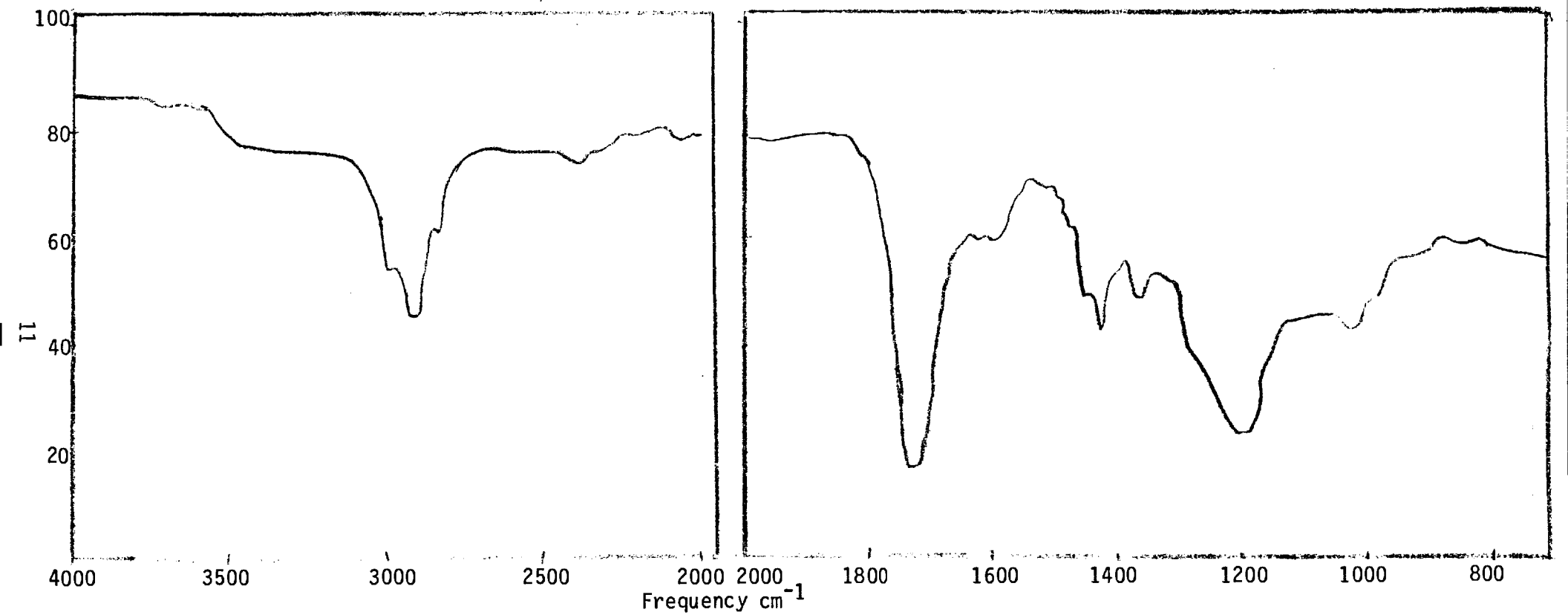


Figure 7. Methylated, Acetylated Aquatic Humics

The methylated-acetylated sample exhibits an infrared spectrum (see Figure 7) which is almost identical to that of the reduced-methylated-acetylated sample shown in Figure 5. This similarity may be due to the presence in the original humic acid sample of relatively few functions which the borane can reduce. A slight narrowing of the low energy side of the carbonyl band may, however, be due to the removal of a broad spectrum of carbonyl groups by the reduction and their replacement by carbonyl groups of a single type—i.e., the acetate functions.

VI. MASS SPECTRAL STUDIES

Mass spectral data were obtained in three general areas, a solids probe analysis of methylated hesperetin, a chemical ionization GC/MS of methylated humic oxidation products, and the electron impact/chemical ionization spectra of three reference compounds.

The reference compounds selected were the methyl esters of benzene 1,2,3-tricarboxylic acid, benzene 1,3,5-tricarboxylic acid and benzene pentacarboxylic acid. The esters were prepared from commercial acids according to standard procedures which were much the same as those used to esterify and protect the acid mixtures produced during the oxidative degradation of humic substances. It is hoped that comparison of these materials with the esterified oxidation products will enable us to decide which of several possible substitution patterns are dominant in the product mixture and that this information can, in turn, be used to make further refinements in the overall structure of aquatic humic substances.

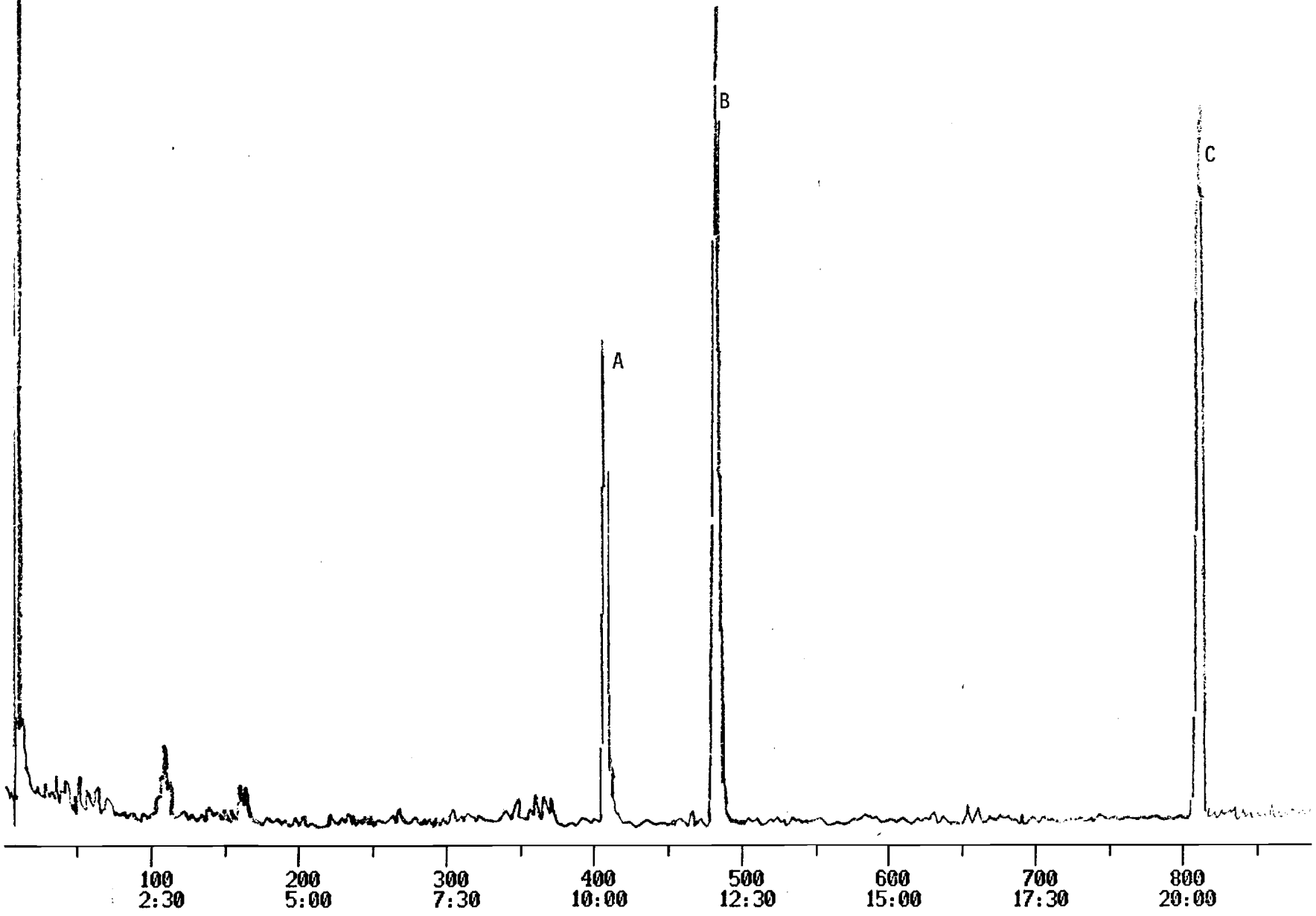
The aforementioned compounds were dissolved in methanol and introduced into the mass spectrometer via the GC interface. The SE-30 capillary column was used in order to assure complete purity of the samples analyzed

by the mass spectrometer. Figure 8 shows a composite of the three total ion chromatograms obtained during the chemical ionization runs. It can be seen that a high degree of separation has been achieved. Gas chromatographic conditions are presented below.

Column:	25 m x 0.8 mm O.D. glass capillary
Stationay phase:	SE-30
Carrier gas:	He
Split flow:	16 ml/min.
Sweep flow:	10 ml/min.
CI Reagent gas:	Methane
Ionizer pressure:	0.41 Torr
Temperature program:	100^{o} -200^{o} at 5^{o}/min.
Transfer line:	248^{o}C
Ionizer:	250^{o}C
Injector:	250^{o}C
Separator:	245^{o}C

The mass spectra of the three compounds are shown in Figures 9-11. In each case, part (a) of the figure is an electron impact spectrum and part (b) is a chemical ionization spectrum.The electron impact ionization spectrum (70 ev) of the methyl ester of benzene 1,2,3-tricarboxylic acid which is presented in Figure 9a indicates that the loss of methoxy to form m/e 221 is a highly favored process—so much so, in fact, that the parent ion is not seen at all—even in the case of chemical ionization with methane. This observation can probably be attributed to the fact that there is a great deal of steric crowding around the ester groups which can be relieved by loss of methoxyl group to form the corresponding acylium ion which is shown below.

Figure 8. Composite Total Ion Chromatogram - Methyl Esters of Benzene 1,2,3-Tricarboxylate(A) Benzene 1,3,5-Tricarboxylate(B), and Benzene Pentacarboxylic Acid(C).



02728778 13:52:00 + 13:57 CALI: 0228 #2 RIC: 1750.
SAMPLE: 1.2.3-TRI(COOCH3) BENZENE

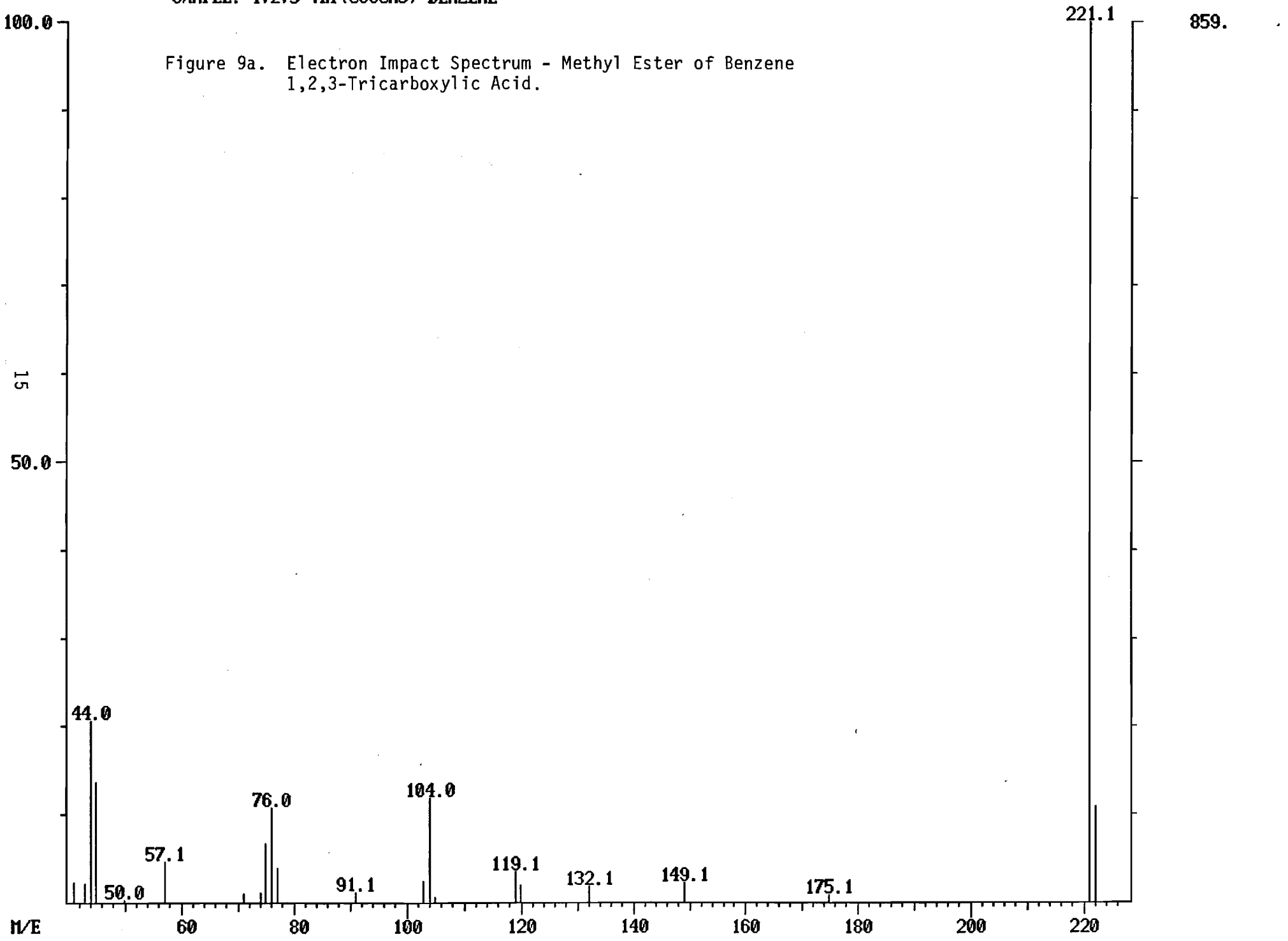


Figure 9a. Electron Impact Spectrum - Methyl Ester of Benzene 1,2,3-Tricarboxylic Acid.

SAMPLE: 1.2.3-TRI(COOCH3) BENZENE

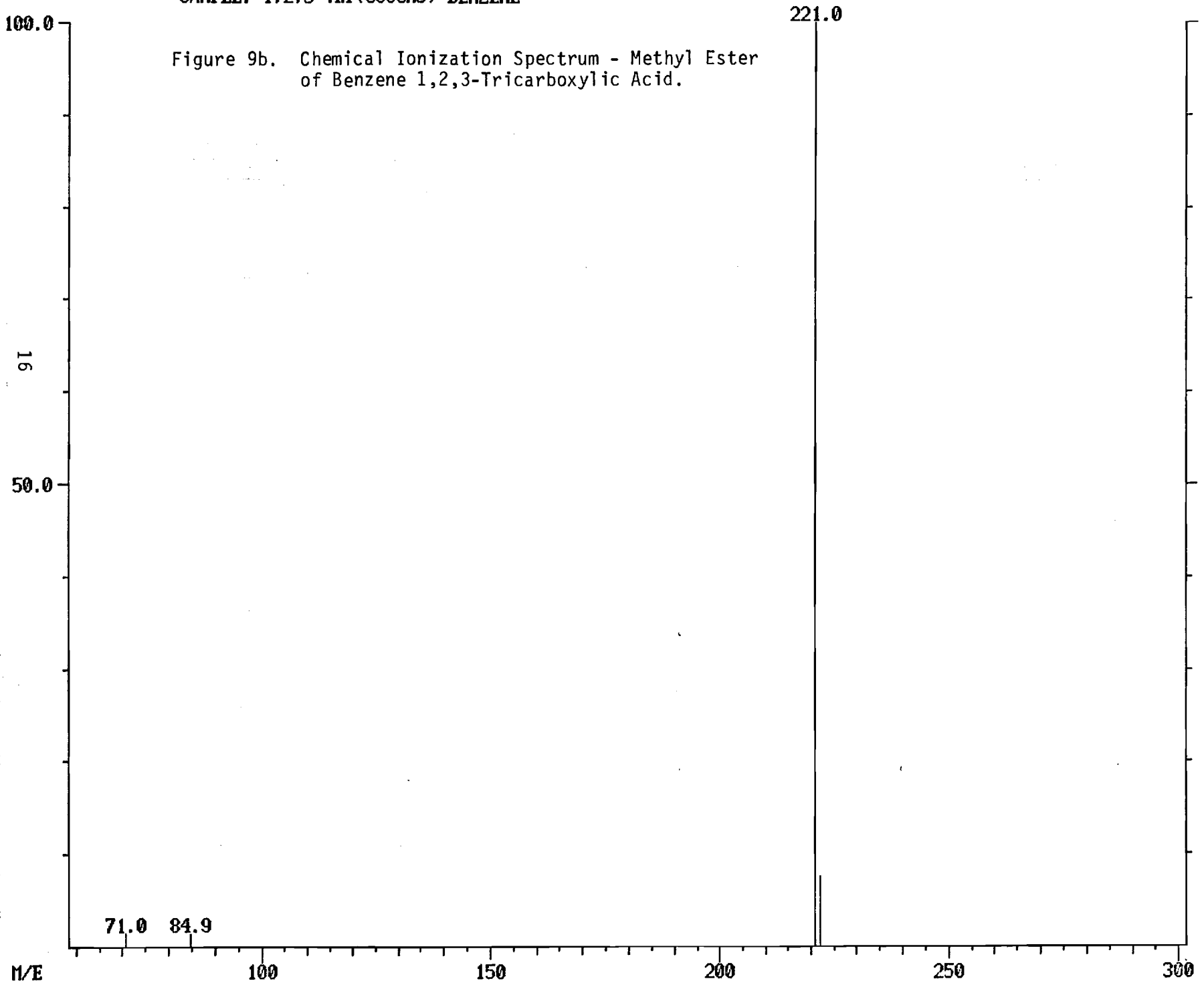
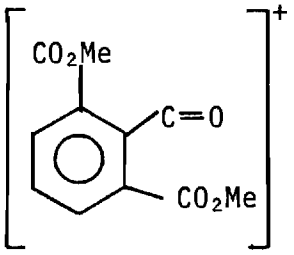


Figure 9b. Chemical Ionization Spectrum - Methyl Ester of Benzene 1,2,3-Tricarboxylic Acid.



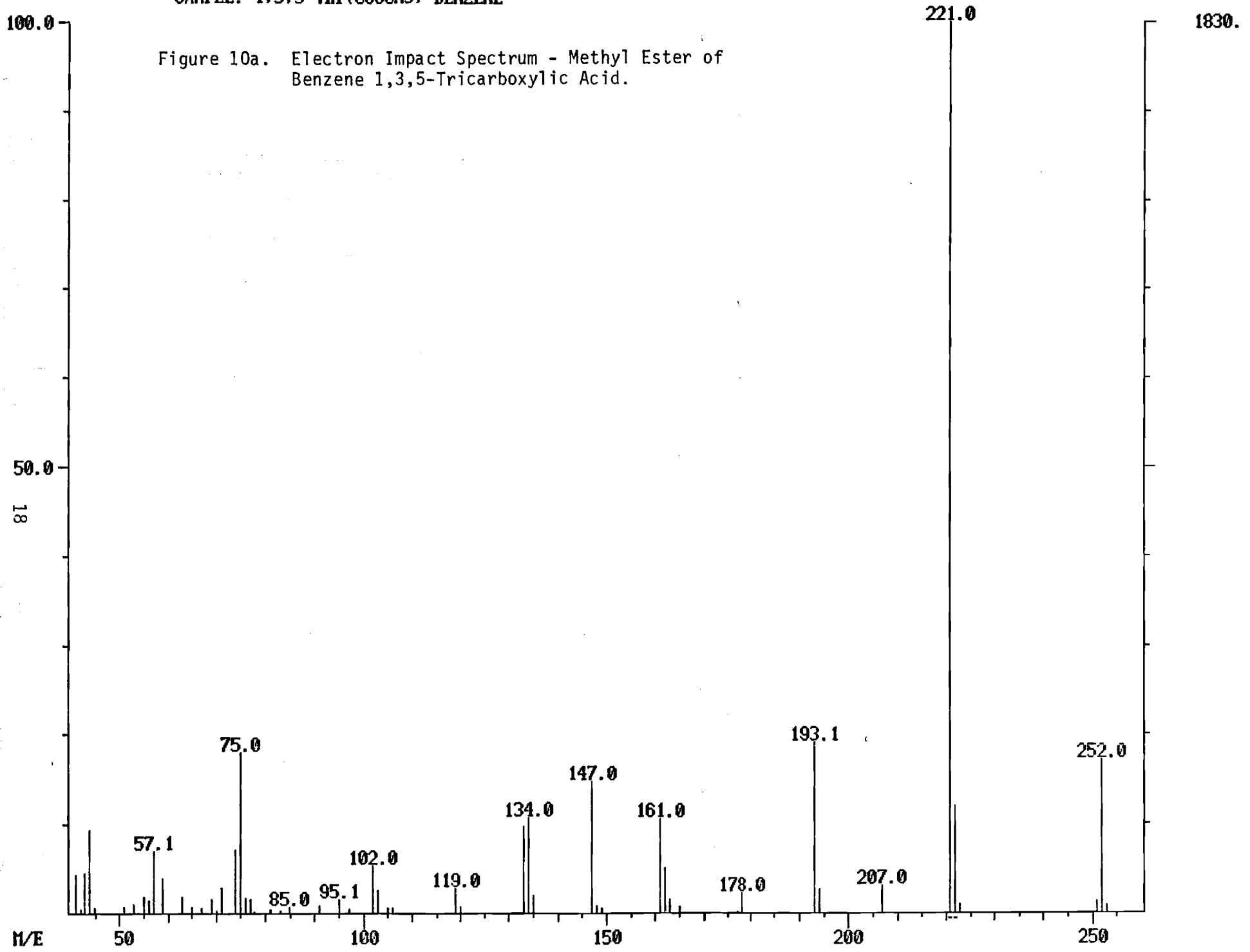
m/e 221

Electron impact ionization of the 1,3,5 isomer (Figure 10a) also results in loss of methoxy as the major fragmentation process. However, there is a significant peak at m/e 252 which is evidently the result of resonance stabilization of the ion permitted by the symmetry of the molecule. This factor coupled with the fact that the separation of the carboxylate groups eliminates steric crowding probably accounts for the observation of the parent ion in this case. The major processes observed in the chemical ionization spectrum of the 1,3,5 isomer (Figure 10b) are the transfer of a proton from CH_5^+ to the molecule to form the M+1 ion at m/e 253, the loss of methoxy to form m/e 221 and the addition of $C_2H_5^+$ to provide m/e 281.

Since the methyl ester of benzene pentacarboxylic acid is an extremely crowded molecule, it is not surprising that the electron impact spectrum (see Figure 11a) shows no parent ion and that the base peak is derived from the parent ion by loss of methoxyl. This peak is also the base peak in the chemical ionization spectrum shown in Figure 11b. In this case, a small peak resulting from the addition of $C_2H_5^+$ to the molecule can also be seen at m/e 397. The retention times of the 1,2,3 and 1,3,5 isomeric compounds relative to the pentamethylester are 0.502 and 0.590

02/28/78 12:58:00 + 15:45 CALI: 0227 #1 RIC: 5576.
SAMPLE: 1.3.5-TRI(COOCH3) BENZENE

Figure 10a. Electron Impact Spectrum - Methyl Ester of Benzene 1,3,5-Tricarboxylic Acid.



SAMPLE: 1,3,5-TRI(COOCH3) BENZENE

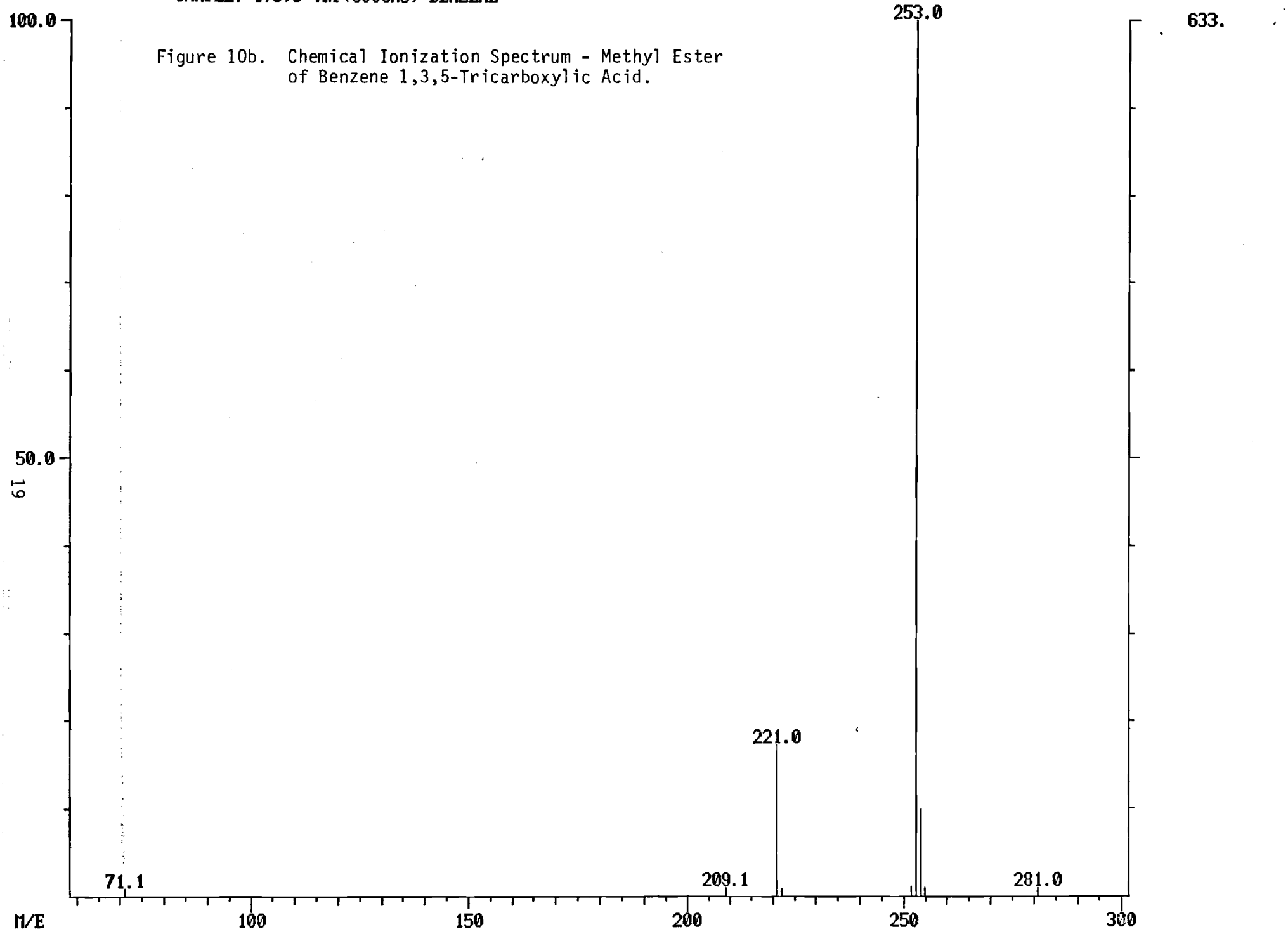


Figure 10b. Chemical Ionization Spectrum - Methyl Ester of Benzene 1,3,5-Tricarboxylic Acid.

02/27/78 17:36:00 + 26:06 CALI: 0227 #1 RIC: 12224.
SAMPLE: 1,2,3,4,5-COOME BENZENE

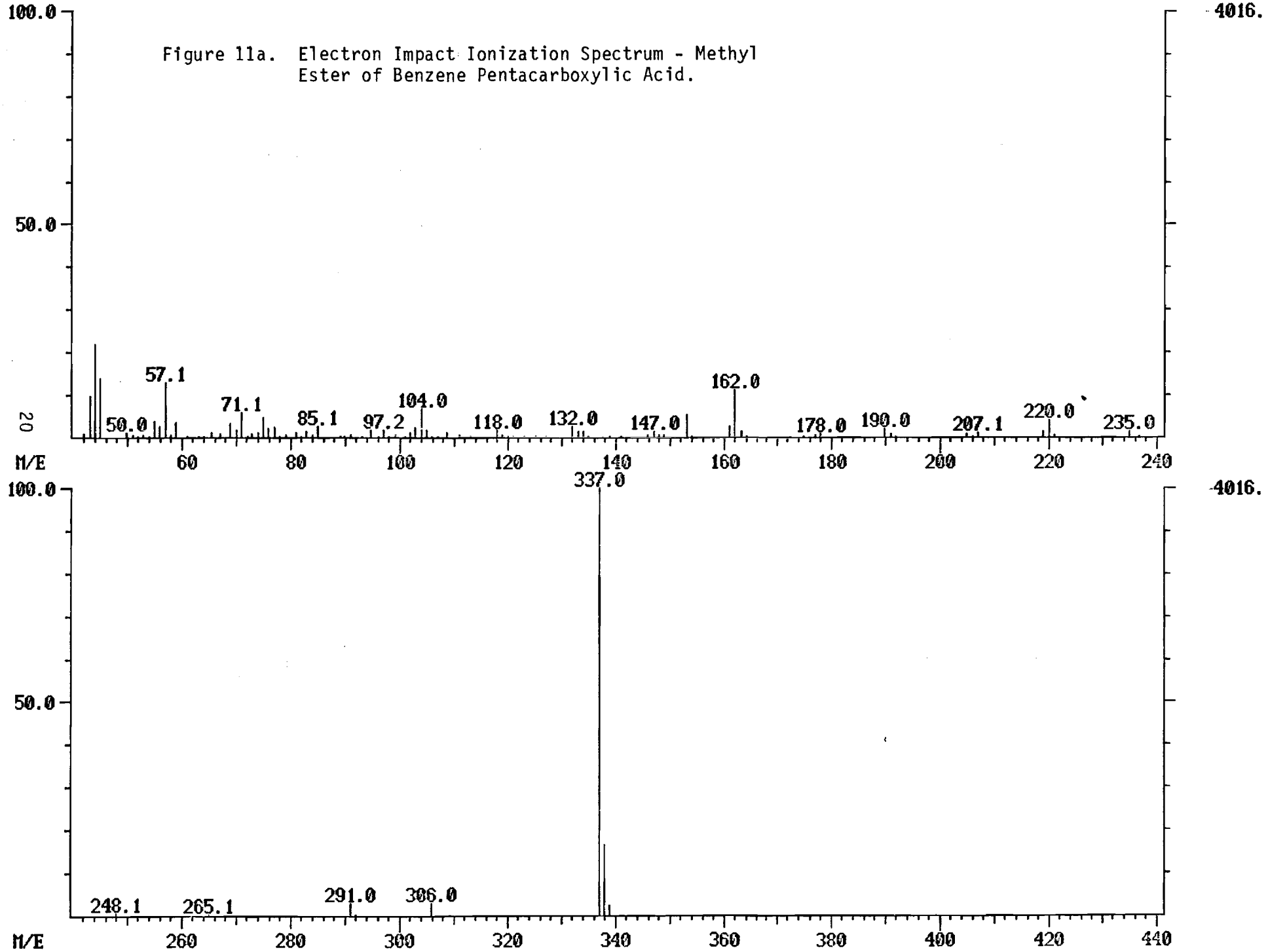


Figure 11a. Electron Impact Ionization Spectrum - Methyl Ester of Benzene Pentacarboxylic Acid.

SAMPLE: 1,2,3,4,5-PENTA(COOCH3) BENZENE

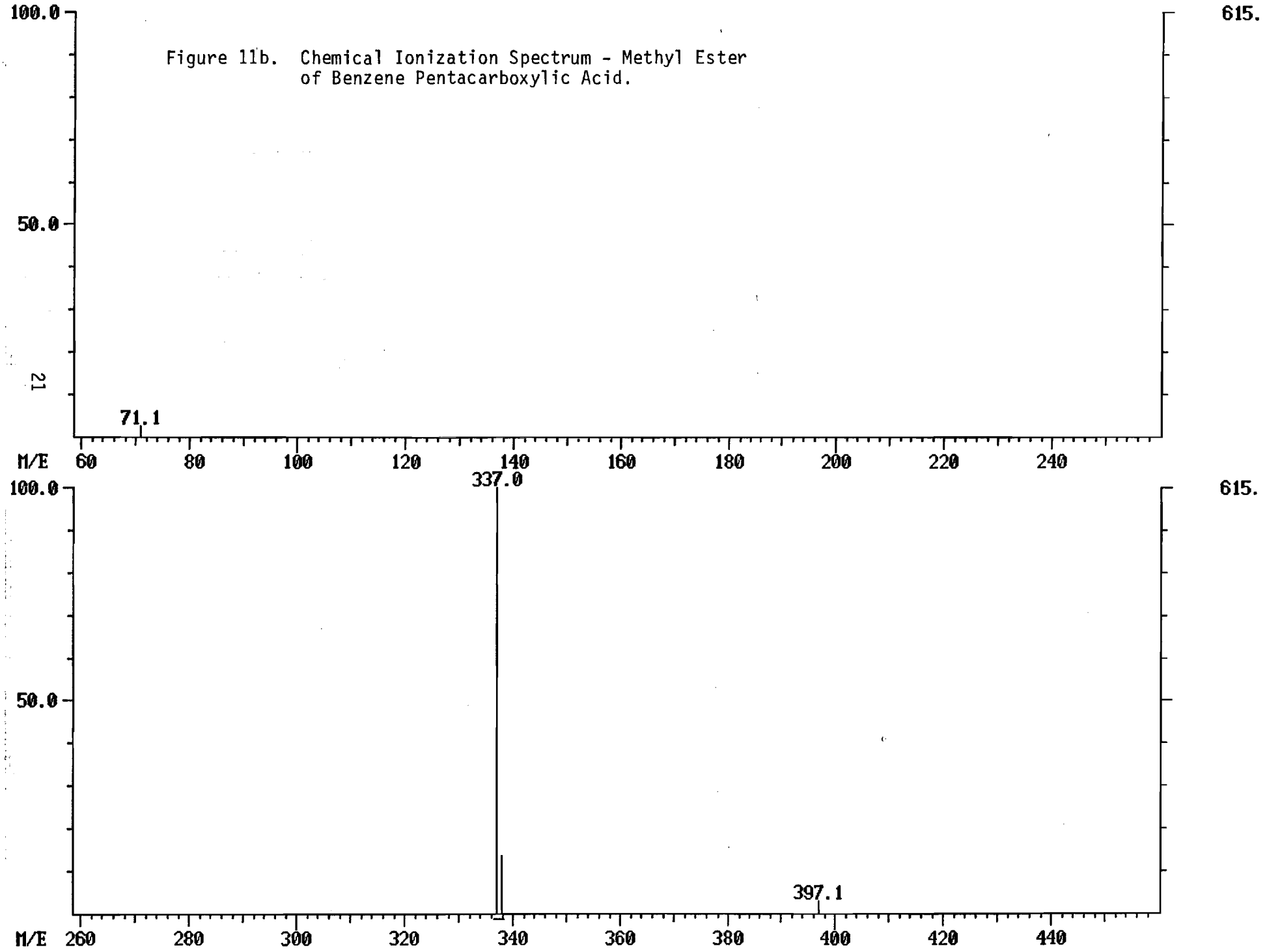


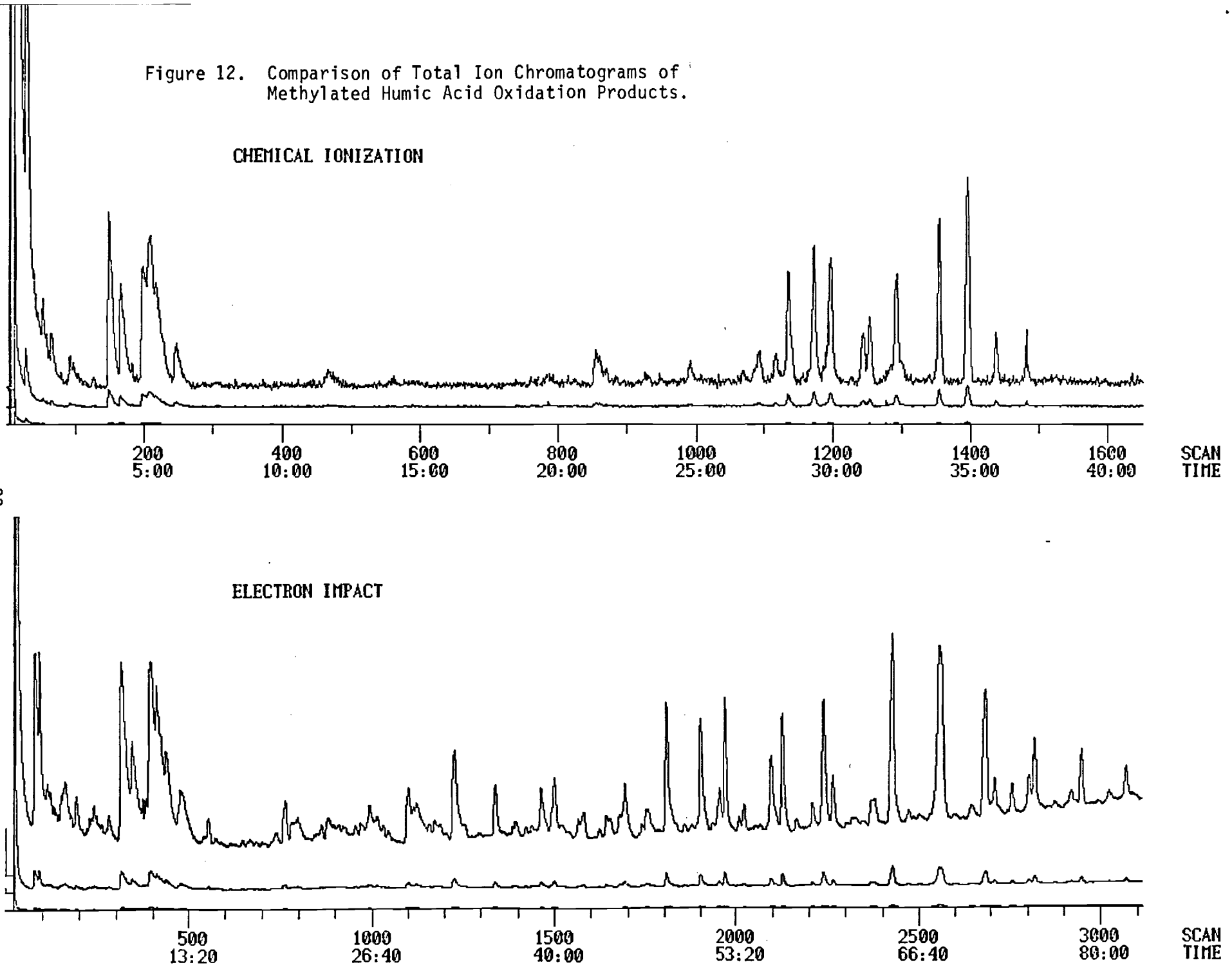
Figure 11b. Chemical Ionization Spectrum - Methyl Ester of Benzene Pentacarboxylic Acid.

respectively. The absolute retention times of the three compounds were 10.17 min., 11.95 min. and 20.25 min.

The methylated permanganate oxidation products derived from aqueous humic matter were subjected to GC/MS using chemical ionization and electron impact techniques. The two total ion chromatograms displayed in Figure 12 are not yet completely complimentary due to the use of slightly different chromatographic conditions for the chemical ionization work. Since these changes in conditions were necessitated by the requirement that make-up gas must be added in the CI case, we plan to change the EI conditions to bring the two results into exact correspondence. Nevertheless, the overall appearance of the two chromatograms is quite similar. Doing the chemical ionization this way (as opposed to using methane as the carrier gas) results in a lowered sensitivity which in turn can bring about the loss of minor GC peaks. Further results will be presented in the next report. The conditions employed for the separation are presented below.

Column:	25 m x 0.8 mm O.D. glass capillary	
Stationary phase:	SE-30	
Carrier gas:	He	
Injector:	250°C	
Transfer line:	260°C	
Separator:	247°C	
Ionizer:	250°C	
	Chemical Ionization	Electron Impact
Split flow:	15.8 ml/min.	45 ml/min.
Sweep flow:	9.7 ml/min.	33 ml/min.
Temperature program	100°-130°C at 1.5°/min.; 130°-220°C at 5°/min; hold at 220°	100°-120°C at 1.5°/min.; hold at 220°
Linear velocity:	250 cm/sec	74 cm/sec

Figure 12. Comparison of Total Ion Chromatograms of Methylated Humic Acid Oxidation Products.



The last of this month's mass spectral studies were carried out using the solids probe technique in the temperature programmed mode. for the analysis of the products resulting from the methylation of hesperetin. The ion mapping technique in which the intensity of a number of separate ions are plotted as a function of time and scan number was applied to serve as an indicator of purity. If more than one substance is present, the relative intensities of the ions in the map will change as a function of time. Since this was found to be the case (see Figure 13), it is evident that the product needs further purification. The lowest trace in Figure 13 is a monitor of the total ion production. A representative scan of the second component of the mixture is shown in Figure 14 and appears to be fully methylated hesperetin. Reaction conditions will be made more rigorous in order to encourage a more complete reaction. The reaction will also be scaled up so that sufficient quantities of material will be available to permit recrystallization.

VII. MINI-PILOT FACILITY

The schematic diagram of the mini-pilot facility which was presented in last month's progress report has been reproduced this month in Figure 14 so that it might be compared with a second schematic shown in Figure 15 which shows only the dimensions of the various components along with the sampling points. It will be noted that some extra components have been added at the bottom of Figure 15. These items are as follows:

1. A 4 liter flask to serve as a clear well. This flask is equipped with an outlet draining into....

SAMPLE: METHYLATED HESPERETIN

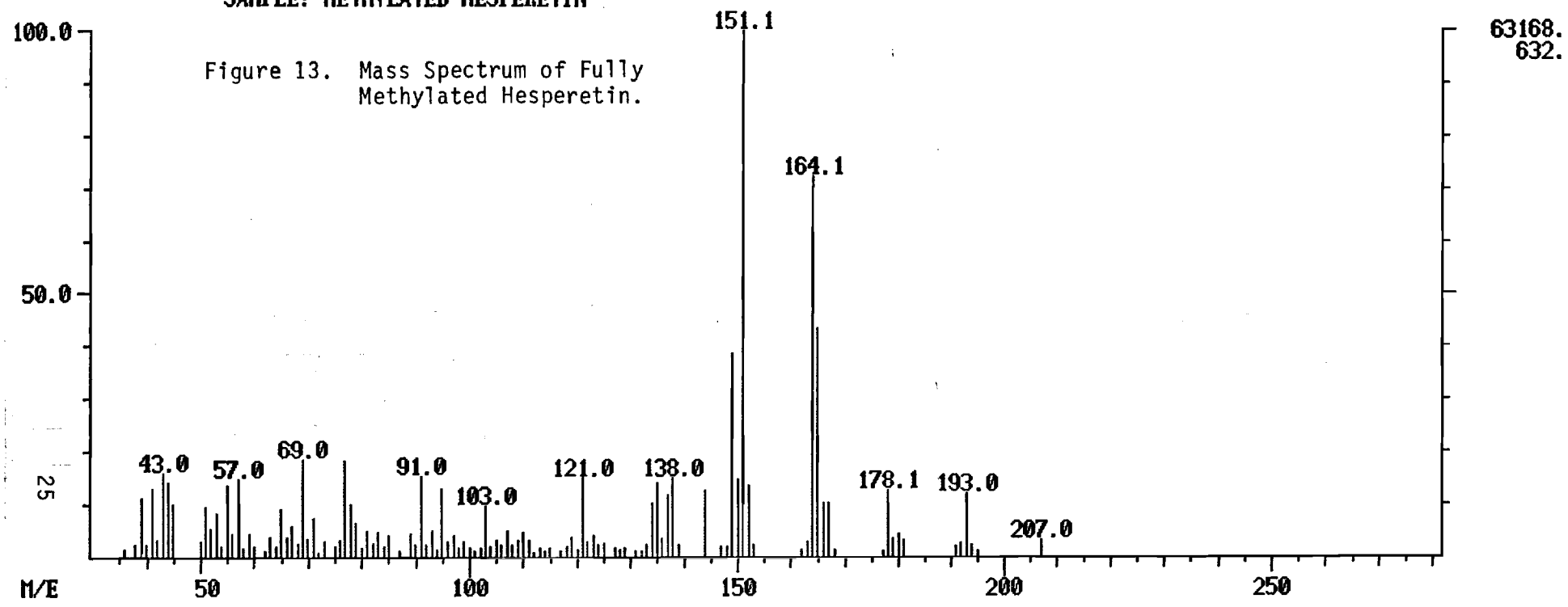
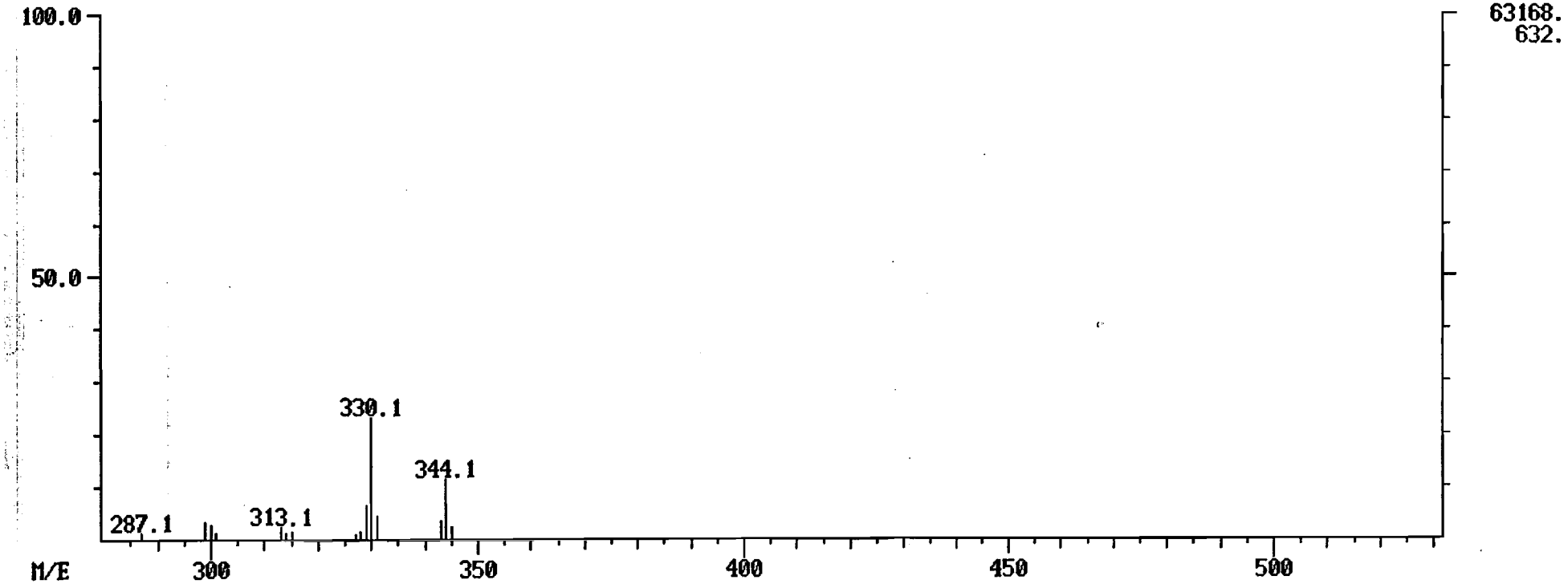


Figure 13. Mass Spectrum of Fully Methylated Hesperetin.



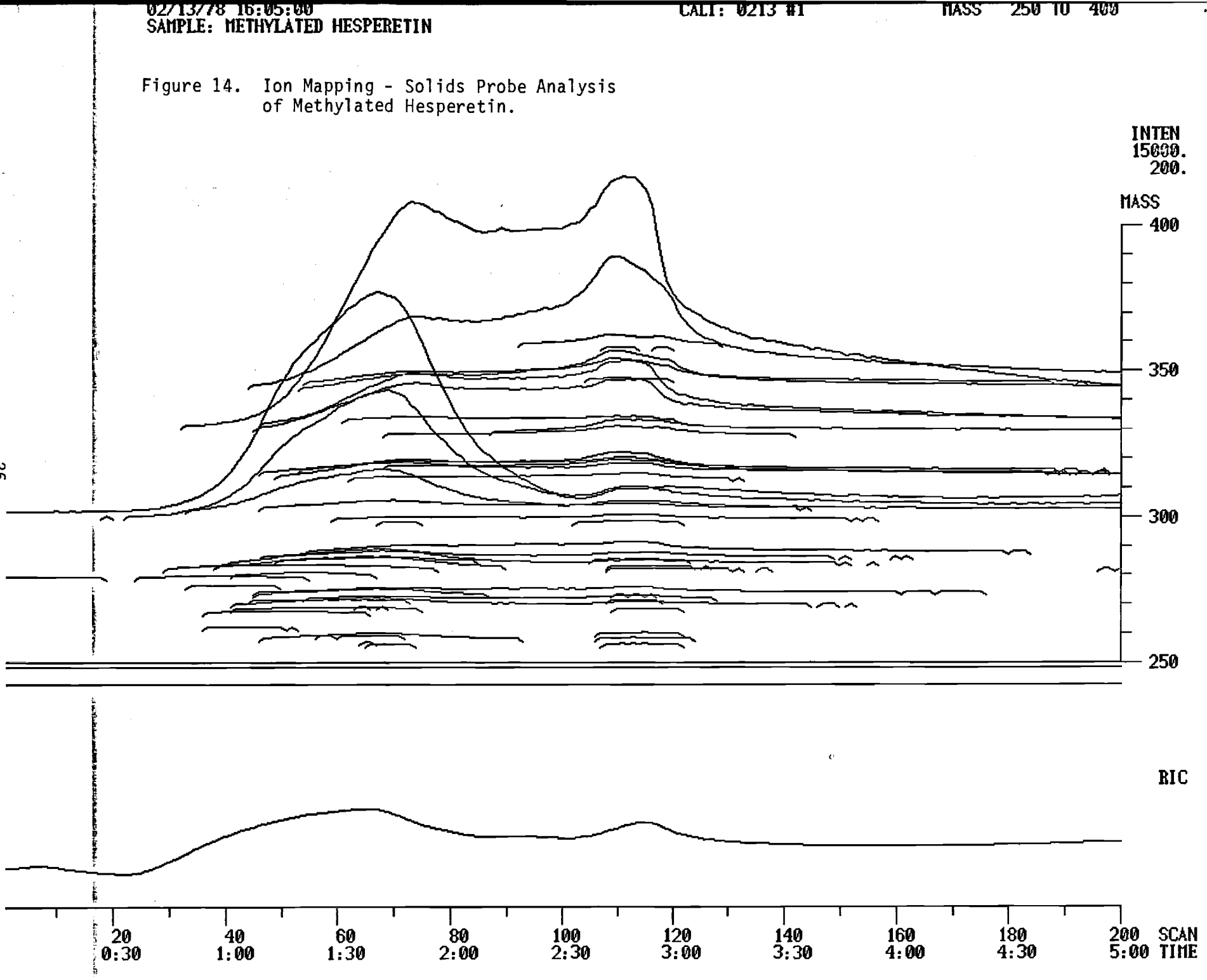


Figure 14. Ion Mapping - Solids Probe Analysis of Methylated Hesperetin.

Figure 15. Schematic - Mini-Pilot Facility

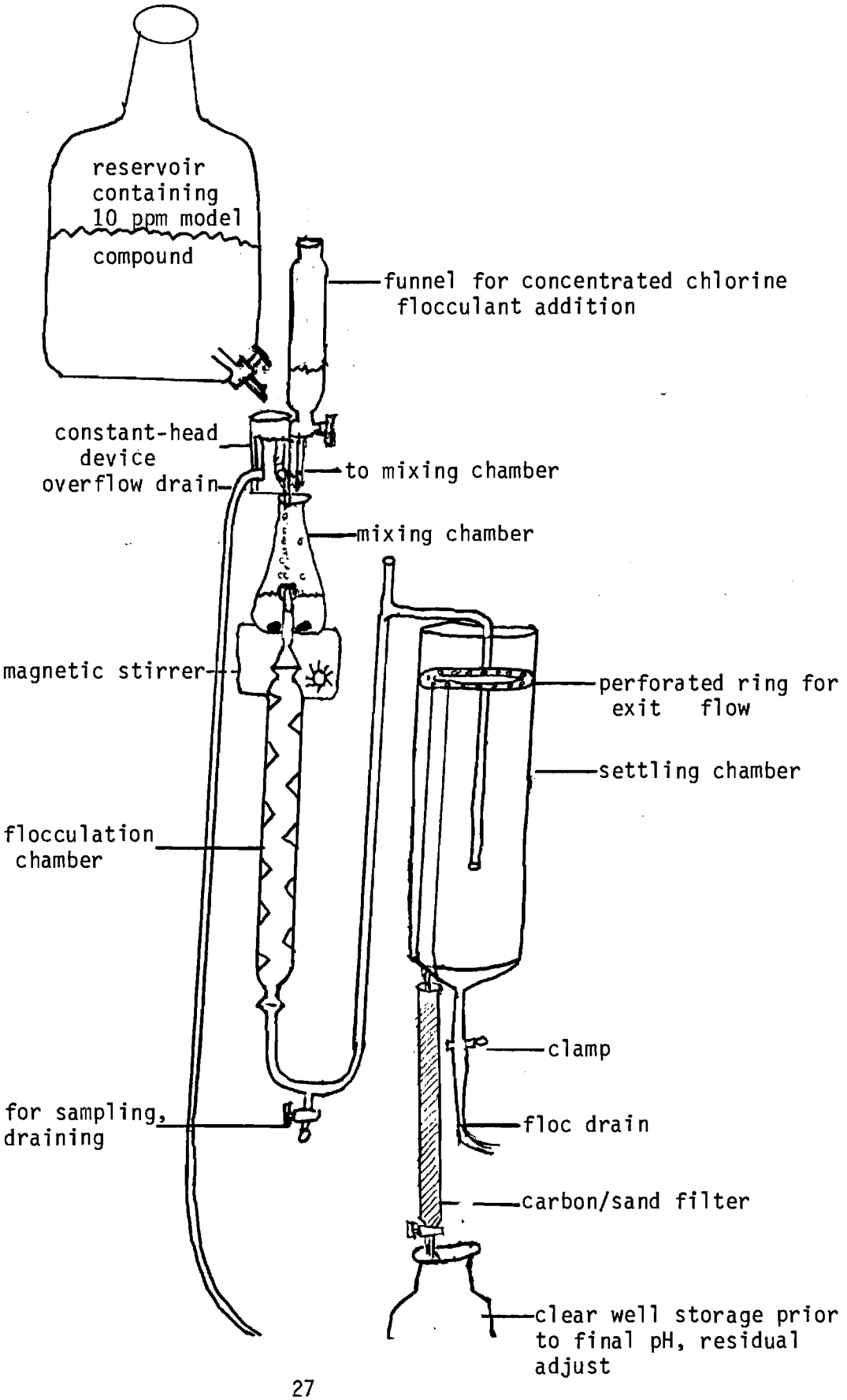
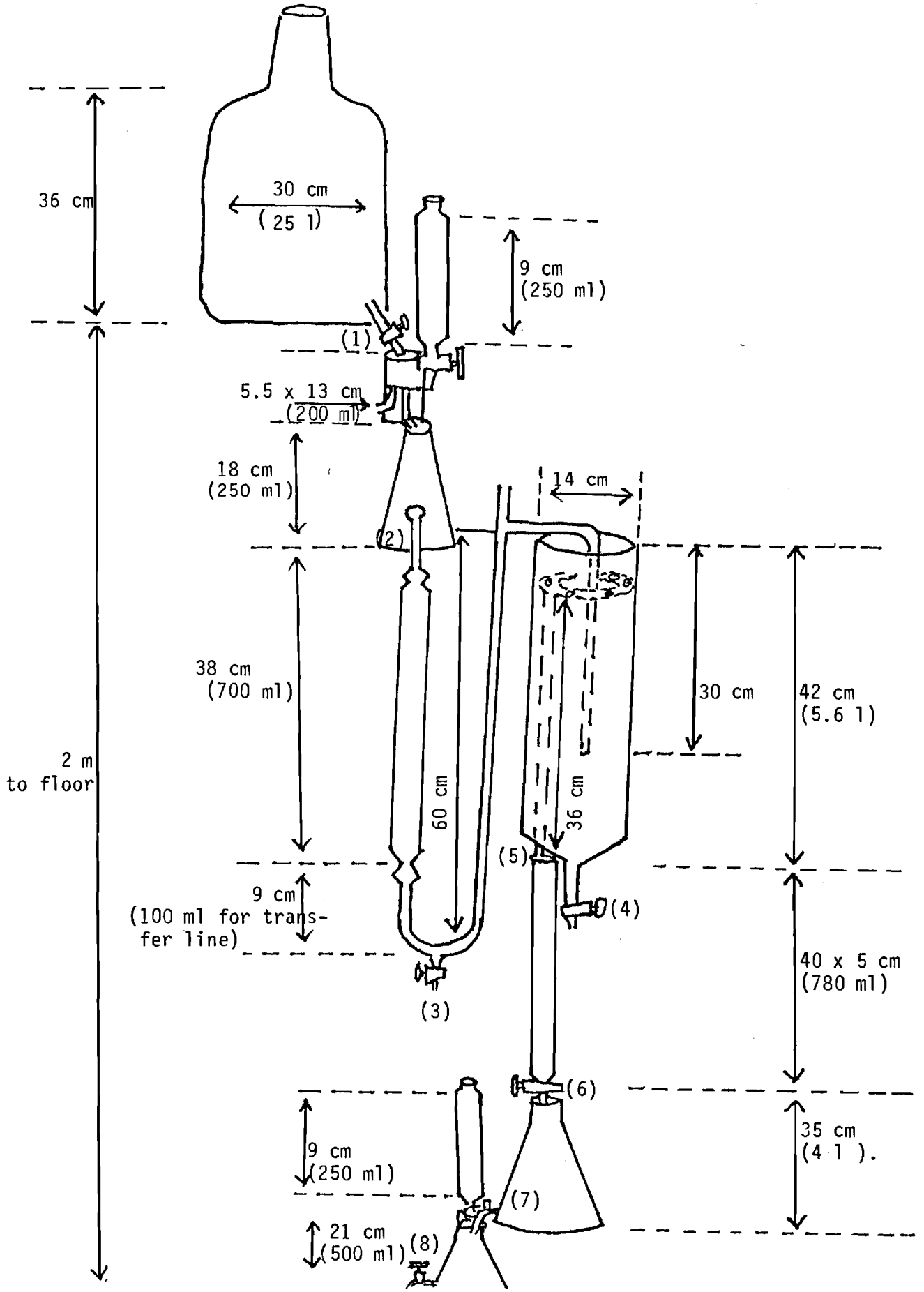


Figure 16. Dimensions and Volumes of Mini-Pilot Facility.



2. A 1 liter flask to serve as a mixing chamber (magnetic stirring) for final residual and pH adjustments. An outlet is provided at the 500 ml mark.
3. A 250 ml additions funnel through which chemicals can be added to the lower mixing chamber.

Samples can be withdrawn at several points:

1. the influent reservoir
2. the exit of the upper mixing chamber
3. the exit of the flocculation device via the stopcock in the transfer line
4. the drain plug at the bottom of the settling chamber
5. between the overflow ring delivery tube from the settling chamber and the top of the column
6. the bottom of the column
7. the exit of the clear well
8. the exit of the lower mixing chamber

Presently we are sampling only the influent reservoir, the settling chamber and the column effluent. As we perfect our analytical methods we shall sample at additional points. It should be noted that the system is modular in design so that components can be resized or rearranged to meet changing needs.

VIII. TOTAL ORGANIC CHLORINE/BROMINE ANALYSIS VIA X-RAY FLUORESCENCE AND NEUTRON ACTIVATION

A 110 mg portion of recrystallized resorcinol was dissolved in 10 ml of phosphate buffer solution (12 g disodium hydrogen phosphate, 60 g potassium dihydrogen phosphate, 100 g sodium metaphosphate made up to a volume of 1 liter with purified water) and added to 10 liters of purified water. Three 5 ml portions of the buffer solution were used to rinse the resorcinol container. The pH of the resorcinol-buffer solution was 6.8. A solution of 700 mg of chlorine in 200 ml of purified water was

prepared by the addition of 1:3 sulfuric acid water to pre-aerated sodium hypochlorite solution. The resorcinol-buffer solution was passed through the mini-pilot facility at a rate of 4 liters per hour while the chlorine solution was added to it at a uniform rate of 100 ml/hr. In this experiment, the sand filter was by-passed. The first liquid discharged from the overflow ring of the settling chamber was found to contain no chlorine residual when tested using the orthotolidine method.

The reaction mixture was allowed to stand for an additional twenty-one hours at room temperature in the settling chamber prior to workup. This solution was also found to contain no residual chlorine and had a pH of 6.0.

A bed (13 mm by 55 mm) of Amberlite XAD-4 resin (purified by extraction with methanol and water) was placed in a glass column and was washed with 200 ml of purified water. The chlorinated resorcinol was passed through the resin at a rate of 25 ml/min. until 6600 ml of eluate had been collected. The resin bed was washed with 200 ml of purified water, the resin removed from the column and dried in air. The dried resin weighed 1.623 g. The treated resin, and an untreated resin sample which served as a control, were submitted for neutron activation analysis for Cl, Br and I. These samples are now in the reactor. Since we had reason to believe that organic acids were being produced which by virtue of their acid strength and water solubility might have passed through the resin bed at pH 6.0, the eluate from the XAD-4 resin bed was diluted with purified water to a volume of 8 liters and the pH adjusted to 2.0 by the addition of 160 ml of 6 N equilibrated hydrochloric acid. The acidified solution was passed through a bed (13 mm x 67 mm) of XAD-2

resin (purified by methanol and water extraction) which had been washed with 200 ml of purified water. The flow rate was 12-15 ml/min. and the total eluate volume was 6600 ml. The resin bed was then washed with purified water (200 ml) until the silver nitrate test for chloride ion was negative. The air dried XAD-2 resin sample weighed 1.946 g. A control sample of XAD-2 resin which was untreated except for washing with a 200 ml portion of purified water weighed 2.911 g after drying in air.

The two XAD-2 samples were analyzed for Cl, Br and I by x-ray fluorescence spectrometry. The untreated control sample showed no detectable halogen concentration. The treated XAD-2 resin sample gave: Cl, 0.023 percent; Br, 0.007 percent. The results of back-calculating to estimate the percentage of applied chlorine found in the water-soluble, strong-acid fraction indicated that slightly less then 0.1% of the applied chlorine was converted to this form. The source of the brominated material is uncertain. The samples have been forwarded to the Georgia Tech nuclear reactor facility for neutron activation analysis.

Since this was basically a preliminary-type experiment designed to test the feasibility of an analytical method and since the initial results were encouraging, a second experiment was carried out which did not involve the use of the mini-pilot facility.

In this case, a solution of 2 g of potassium dihydrogen phosphate and 10 g dipotassium hydrogen phosphate in 10 liter of purified water was purged with a stream of nitrogen for 20 minutes to remove volatile components. The pH of the solution was 7.57; it was divided into two 5 liter portions.

A solution of 55 mg of recrystallized resorcinol was added to one of the 5 liter portions of buffer solution. A solution of 380 mg of chlorine (freshly generated from nitrogen-purged sodium hypochlorite solution) in 50 ml of purified water was added to each of the 5 liter portions of buffer solution. The solutions were mixed by shaking the containers several times during a 30 minute period. The chlorination reaction was allowed to proceed at 22-24^{o}C over a period of 14 hours. At the end of this period, no residual chlorine was present in the resorcinol treated solution. The buffer solution containing only chlorine showed a residual of 30.7 mg per liter. The two solutions were each treated with 0.500 g portions of sodium sulfite. After this treatment, both solutions showed no residual chlorine.

The pH of the two solutions was adjusted to 1.9 (from 7.45) by the addition of 45 ml of concentrated sulfuric acid.

Two XAD-2 resin beds were prepared as slurries using precleaned (Sohxlet extraction of commercially supplied resin with methanol and water) resin. The resin beds were 13 mm x 67 mm in size. After placing pyrex glass wool plugs on the top of the resin beds, the resin was washed with 200 ml of purified water. The two 5 liter volumes were passed through the two resin beds at a flow rate of 10-15 ml per minute. The resin beds were rinsed in portions with a total volume of 500 ml for each batch of resin. At this stage of rinsing, the pH of the rinse water was the same (5.62) as that of the purified water. The two portions of resin were placed on sheets of Whatman No. 1 filter paper, covered with another sheet, and dried in air for 16 hours at room temperature. The dry resin which had been contacted with the control solution weighed

2.522 g and the resin which had been contacted with the solution containing resorcinol weighed 2.152 g. The two resin samples were thoroughly homogenized and submitted for analysis by x-ray fluorescence spectroscopy. The analytical work is now in progress.

IX. REACTION OF HESPERETIN WITH CHLORINE IN THE MINI-PILOT FACILITY

A solution of 100 mg of purified hesperetin in 10 l of high-purity water was prepared and adjusted to pH 7.0 by the addition of roasted[8] potassium dihydrogen phosphate and disodium hydrogen phosphate. It was found to be necessary to first dissolve the hesperetin in 200 ml of 0.25 N sodium hydroxide in order to speed up the dissolution process.

Commercial soium hypochlorite (100 ml) was pre-purged with nitrogen in order to remove any volatile haloorganic contaminants. Subsequent addition of 1:3 sulfuric acid:water produced gaseous chlorine which was swept into a solution of high-purity water by a stream of nitrogen. A portion of the resulting chlorine solution was diluted and titrated with ferrous ammonium sulfate (Palin method)[9] in order to establish its strength. The results indicated that the concentration of chlorine was 4.0 mg/ml. This concentrated solution (200 ml) was added to the hesperetin solution (8.6 l) as it flowed into the mixing chamber of the mini-plant. The flow rates of chlorine concentrate and hesperetin were adjusted so that both reagent solutions would be exhausted at the same time - i.e., 86 mg hesperetin = 800 mg Cl_2. The color of the hesperetin solution changed to yellow in the mixing chamber and a small amount of yellow solid separated out in the flocculation chamber. No chlorine residual was observed in the initial overflow from the settling chamber. In the future, larger doses of chlorine will be applied. The flow rate of hesperetin containing influent water was 1.4 l per hour.

The yellow solid which had separated out on the walls of the mixing chamber was transferred to a filter paper cone and air dried to provide less than 1 mg of solid. This material has been stored for future characterization.

The hesperetin solution which remained in the influent reservoir (1.4 l) was treated with 8.25 g of roasted sodium carbonate to bring the pH to 10.2. The solution was then extracted with glass-distilled pentane (4 x 50 ml). The combined pentane extracts were dried over roasted sodium sulfate and concentrated in a Kuderna-Danish apparatus to a volume of 4 ml.

The pentane-extracted hesperetin solution was subsequently acidified to pH 1.3 with 10 ml of sulfuric acid and extracted with purified ethyl ether (3 x 100 ml). The combined ether extracts were backwashed with one 50 ml portion of purified water. The ethereal solution was dried over roasted anhydrous sodium sulfate and concentrated in a Kuderna-Danish apparatus to a volume of 4 ml.

A 3.45 l portion of chlorine-treated hesperetin solution which was collected after passage through the sand filter was treated with 14.42 g of roasted sodium carbonate, to provide a pH of 10.0, and extracted with glass-distilled pentane (4 x 50 ml). In this case, the sand used in the filter was Ottawa type, 20-30 mesh, roasted prior to use. The pentane extracts were concentrated in a Kuderna-Danish apparatus to a volume of 4 ml.

The above solution was then adjusted to pH 1.3 by the addition of 20 ml of sulfuric acid and extracted with glass-distilled ethyl ether (3 x 200 ml). The combined ether extracts were backwashed with one 100 ml portion of purified water and dried over roasted anhydrous sodium sulfate. The yellow ethereal solution was concentrated in a Kuderna-Danish apparatus to a volume of 4 ml.

A second run was carried out in much the same manner. In this case, the initial pH was 7.42, the hesperetin concentration was 10 ppm, the reservoir charge was 10.0 l, the total chorine dosage was 1,541 mg which was reacted with 68 mg (6.8 l) of the hesperetin solution. Flows were adjusted so as to maintain a constant chlorine dosage throughout the course of the treatment (9 hours). At the end of the addition, the sand filter eluate had a pH of 7.17 and showed no chlorine residual.

The chlorine-treated hesperetin solution collected in the settling chamber was checked for chlorine residual after a 24 hour contact time. The residual was found to be 8.4 mg/l. The chlorine residual was then quenched by the addition of 100 ml of sodium sulfite.

The following samples (125 ml in volume) were stored in septa closure bottles for future analysis: (1) untreated hesperetin solution, (2) sand filter eluate and (3) the chlorinated hesperetin solution which had been collected in the settling chamber.

The untreated hesperetin solution remaining in the reservoir had a volume of 3.2 l and a pH of 7.24. The usual pH adjustment, solvent extractions and concentrations were carried out as before.

Since all other materials used in these experiments were subjected to roasting in order to remove organic contaminants, the only possible mode of introduction of such contaminants would be with the chlorine itself. While pre-purging of the hypochlorite solution should greatly reduce this possibility, it may be that the sulfuric acid which is added to generate the chlorine from the hypochlorite:

1. contains volatile haloorganics,
2. converts haloorganic acid salts in the hypochlorite to the corresponding volatile acids,
3. decomposes otherwise stable intermediates leading to the formation of volatile haloorganics.

Control experiments have therefore been carried out to investigate this possibility. In one such case, a concentrated solution containing 340 mg of chlorine in 250 ml of highly purified water was prepared in the usual manner. The chlorine was converted to chloride by application of 630 mg roasted sodium sulfite. The volume of the resulting solution was adjusted to 500 ml with high-purity water and the pH was raised to 10.0 by the addition of 2.3 g of roasted sodium carbonate. The solution was then extracted with glass-distilled pentane (4 x 50 ml). The combined pentane extracts were dried over roasted anhydrous sodium sulfate and concentrated in a Kuderna-Danish apparatus to a volume of 4 ml. The pentane-extracted solution was acidified to a pH of 1 with 10 ml of sulfuric acid and extracted with one 100 ml and two 50 ml portions of glass-distilled ethyl ether. The combined ether extracts were backwashed with one 50 ml portion of purified water and dried over roasted sodium sulfate. The dried ether solution was concentrated in a Kuderna-Danish apparatus to a volume of 3 ml After the pentane and ether extracts had been concentrated, bottled and stored in a freezer, the remaining aqueous solution was extracted with "Spectranalyzed" chloroform (3 x 50 ml) which was similarly dried, concentrated and stored. (It will be recalled that our special porous anode EC-type gas chromatographic detector allows us to analyze extracts prepared in halogenated solvents.)

X. LIQUID CHROMATOGRAPHIC STUDIES

A Vydac C-18 reversed phase column 25 cm x 3.2 mm was used throughout the work described in this section. The apparatus employed for this work was a MSI Model B-550 liquid chromatograph equipped with a gradient-elution chamber, a stop-flow injector, a fixed-wavelength UV detector with a scanning accessory. The two detectors were connected in series. Results were recorded using a two-pen Linear Instruments recorder. Solvents were degassed prior to use. Flows were maintained in the vicinity of 1.5 ml per minute unless otherwise stated.

Retention volumes were established for trichloroacetic acid using a solution of several ppm in methanol/water (80/20). The injection volumes unless otherwise stated, were 0.6 µl. The first solvent system investigated was 65/35 methanol-water (isocratic). The observed retention volumes were 1.4 ml. A typical result is shown in Figure 17. Detection was at 254 nm and 210 nm. It is interesting to note that a second more strongly retained peak (probably due to the methyl ester), began to "grow in" over the course of several days. This observation suggests that analysis of acids in alcoholic solvents should be carried out immediately. The use of other solvent systems such as acetonitrile, which cannot react with free acids, has also been considered but has not yet resulted in improved separation.

Dichloroacetic acid was observed to have a retention volume of 1.6 ml under the aforementioned conditions. This is not considered to be sufficiently different from that of trichloroacetic acid to suit our purposes. A linear gradient elution progressing from 100% water to 100% methanol did result in the production of multiple peaks but was still

deemed unsatisfactory since the late-eluting peak was poorly defined. Work is continuing in this area.

A recent literature citation[10] describing a method for the purity determination of hesperetin by gradient-elution, high pressure liquid chromatography inspired some efforts in this area. A 10 to 100% methanol in 0.03 M potasssium dihydrogen phosphate buffer system was employed. The results were in excellent agreement with the literature—even to the extent of observing a slight impurity (probably isosakuranetin) in the sample provided by Coca-Cola (see Figure 18). The observed retention times and volumes were 4.2 min. and 6.3 ml, respectively. A second sample obtained from the Pasadena, California USDA laboratory showed no evidence of this impurity. These conditions will be employed as a starting point for the analysis of the product mixtures from the model compound studies.

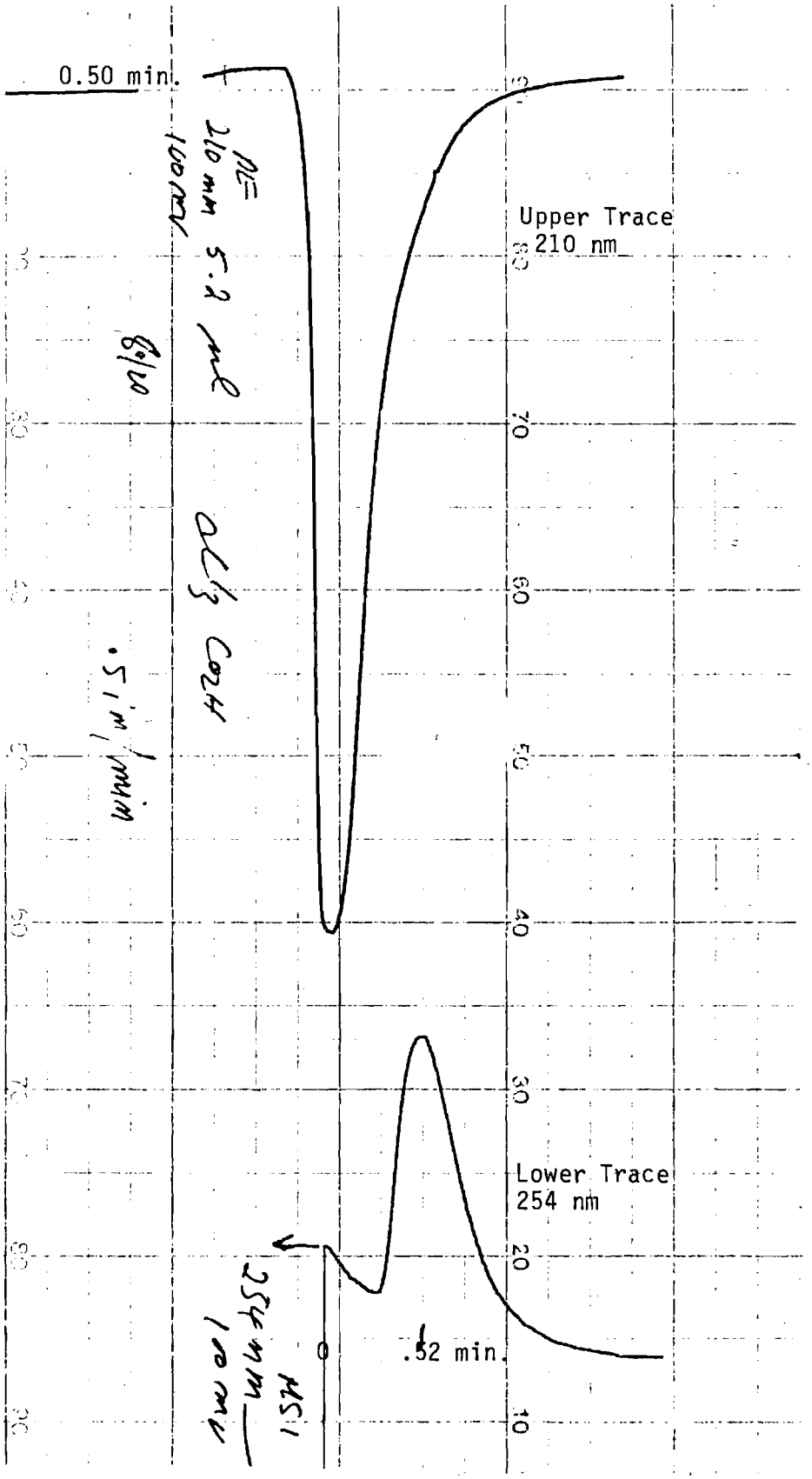


Figure 17. Trichloroacetic Acid (methanol/water-65/35 isocratic).

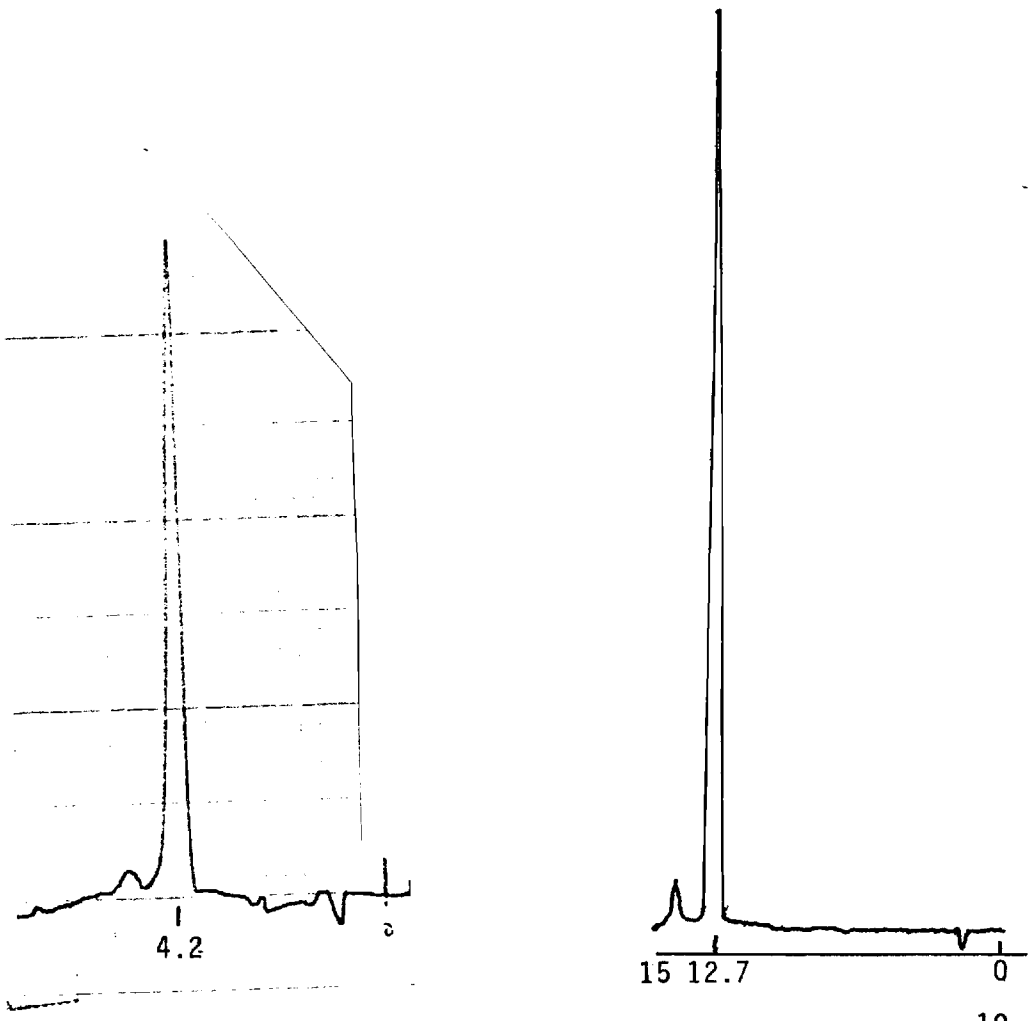


Figure 18. Purity of Hesperetin by LC.

XI. SUMMARY AND PLANS

The total acidities, phenolic acidities and carboxylate acidities of two samples of aquatic humic material were estimated. It was found that the differences between the two samples were not great and that the phenolic and carboxylate acidities were nearly equal. Spectral studies on aquatic humics which had been subjected to reduction, methylation and acetylation indicated that unlike soil-derived humic materials, aquatic humics can be methylated to the point at which they become soluble in organic solvents without resorting to drastic procedures. These standard methylations do leave unreacted alcoholic groups behind which can subsequently be acetylated to the point that no-OH functions can be detected by infrared spectroscopy. Reduction of aquatic humics with trimethylamine-borane does not appear to produce extensive structural changes.

GC/MS studies with the methyl esters of benzene 1,2,3 tricarboxylic acid, benzene 1,3,5 tricarboxylic acid and benzene pentacarboxylic acid show that these compounds are well separated under the conditions of analysis. The EI and CI spectra both provide useful information with the latter being considerably simpler. It is evident that the loss of methoxyl, particularly in the crowded isomers is a dominant process even in the CI spectra. A comparison of the total ion chromatograms derived from methylated humic acid oxidation products using EI and CI methods indicates that individual spectra can be correlated to provide additional information. A mass spectral analysis of the products obtained from the treatment of hesperetin with diazomethane indicates that, although some complete methylation has taken place, more drastic conditions will be necessary in order to assure a more complete reaction.

A schematic of the mini-pilot facility showing volumes and dimensions has been provided. The clear well reservoir has been fitted with an outlet leading to a second mixing chamber so that chemicals can be added at this point for final pH and residual adjustment. Two studies with the mini-pilot facility have been carried out using hesperetin as a model compound. Samples have been taken at key points, fractionated and concentrated. Analysis of this material is in progress.

Studies designed to develop an analytical method for total organic halogens using resin trapping followed by x-ray fluorescence and neutron activation analyses are under way. Preliminary data from studies using resorcinol as a model compound are encouraging.

Liquid chromatographic methods are being developed for the analysis of organic haloacids and hesperetin. Excellent results have been obtained in the latter case. An anion exchange column has been ordered to speed progress with the analysis of the acids.

Future work will include studies on methods for the isolation and subsequent analysis of halogenated, water-soluble acids such as might be generated during the water treatment process. Isolation of volatile acids is now in progress using steam distillation and will be followed by GC analysis on an FFAP/H_3PO_4 column using our novel porous anode EC-type detector for quantitation.

Preliminary studies are planned using iodine plus hesperetin. These will be followed by experiments using aquatic humic matter. River humics will also be put through the mini-pilot facility for the first time using chlorine as the disinfectant/oxidant.

We have not yet had the opportunity to carry out preliminary experiments using chloroform together with chlorine in basic or neutral solutions in an

effort to detect carbon tetrachloride as a reaction product. Conversations with Dr. E.S.K. Chian at the U. of Illinois have added further support to his suggestion that carbon tetrachloride is being produced by the reaction of chlorine with humic matter.

We have begun a series of experiments using ozone generated by corona discharge in an all-glass system designed to test the action of O_3 on purified resorcinol under a variety of conditions. The key experiments will be concerned with the action of ozone on resorcinol in the presence of added bromide. Since ozone is a stronger oxidant than bromine (or iodine, or chlorine for that matter), it may be that Br_2 can be generated rapidly enough to allow for reaction with the resorcinol before it is blown out of the reaction vessel. It is also possible that trihalomethanes can be produced by ozone but are lost due to the continuous purging action of the ozone-containing gas stream. Therefore, we shall look for non-volatile halogenated products using a resin absorption technique. If time permits and if the results are encouraging, this work may be expanded to include photochemically-produced ozone.

Internal consultation with Dr. S. B. Smith has resulted in the preliminary design of activated-carbon experiments in the sense that a number of factors have been discussed which might be evaluated. These are listed below.

1. Compounds tested
    a. Hesperetin
    b. Aquatic humics
2. Reagents employed for disinfection
    a. Chlorine
    b. Iodine
    c. Chlorine dioxide

3. Type of carbon

   a. Low activity, low capacity, small pore size such as MWT or WVW - this type may be effective at holding effluent levels to very low values, but would have relatively short usage times
   b. High activity, high capacity, large pore size such as Fitrasorb-400 or WVG - this type would be expected to have a relatively long lifetime but might permit low levels of breakthrough almost immediately.
   c. Reactivated spent carbon - Dr. Smith has equipment to perform controlled reactivations in his laboratory. We may therefore wish to include carbon of this type if the sponsor so desires.

4. Mesh size of carbon - only two types would seem to be appropriate for this type of work

   a. U.S. sieve 8 x 30 (coarser)
   b. U.S. sieve 12 x 40 (finer)

5. Placement of the column in the system

   a. At the beginning - prior to the addition of chlorine
   b. At the end - after settling

6. Depth of the carbon bed

   a. Very thin
   b. On scale with current practice
   c. Thick

The parameters measured would be trihalomethanes and total organic halogen. The points of measurement have already been described in earlier sections. Observations before and after the column will be particularly useful. It will not be possible to examine all of the factors within the present scope of work. Therefore, we would request that the sponsor assist in setting priorities.

XII. REFERENCES AND FOOTNOTES

1. Omitted during revision.

2. Omitted during revision.

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4. G. Gran, Part II, Analyst, 77, 661 (1952).

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6. R. L. Wershaw and D. J. Pinckney, Science, 199, 906 (1978).

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8. "Roasting" is carried out at 400^{o}C in a muffle furnace immediately prior to use in order to burn off any organic contaminants which might otherwise confuse the experimental results.

9. Standard Methods, 11th ed., APHA-AWWA-WPCF, New York, (1960), p. 99.

10. C. T. Seitz and R. E. Wingard, J. Agric. Food Chem., 26 278 (1978).

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

April 11, 1978

by

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DISCLAIMER

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I. PERSONNEL

Ms. J. Lange and Dr. J. Carden have been added to the laboratory staff as of the first week in April. Ms. Lange is a student co-op (full time) while Dr. Carden comes to us from Bogazici University in Turkey where he was very active in environmental analytical chemistry. While neither were aboard during the past reporting period, it is envisioned that both will play a contributory role in future work on this project.

II. EQUIPMENT

As of this writing, all equipment is operating smoothly as it has been throughout the reporting period. A new disc drive has been installed on our GC/MS unit. Since our own staff had correctly diagnosed the problem in its early stages before it resulted in an instrumental shut down, a replacement unit was on hand when the original expired. As a result, the instrument was only down for four hours.

A U-tube was added to the filter column of the mini-water treatment facility in order to maintain the water level above the surface of the bed so that a more even flow through the bed can be maintained.

III. ESTIMATION OF TOTAL ACIDITY OF AQUATIC HUMICS

The results of duplicate analyses of the total acidity of aquatic humics were carried out using the procedure described in the last monthly progress report. Two samples each representing a different month were examined. The results are presented below

Sample	Total Acidity in meq/g	
M/30	11.11 11.32	avg. 11.21
M/39	10.91 10.45	avg. 10.68

IV. ESTIMATION OF CARBOXYL GROUPS IN AQUATIC HUMICS

A new, promising approach to the estimation of this property is being developed in Dr. Reuter's laboratories. This method involves the displacement of acetic acid from 0.1 N calcium acetate solution by aquatic humic carboxyl functions in the manner described in earlier reports. In this case, however, the equilibrated solution is filtered through a membrane (CPM-10) designed to reject polymeric material which is believed to be the cause of the indistinct inflection points observed in the subsequent titrations. While some colored material was observed to pass through this particular type of membrane, the titration curve was much improved over those obtained by the old method (see Figure 1). Other membranes (Amicon UM05) will be used in an effort to further improve the method. The results obtained by the new method are (5.11 meq/g vs. 5.5 meq/g) not significantly different. The improvement lies in the reliability of the results which are now based on a more distinct inflection point.

V. PERMANGANATE OXIDATION OF AQUATIC HUMICS

As indicated in earlier reports, a milder version of the oxidative degradation with alkaline permanganate is needed so that more of the original humic structure is preserved. In order to further these efforts, a pair of reactions were carried out on the 1.0g scale. One of the mixtures was treated in the usual way and provided 181 mg of methylated product. The second reaction mixture was methylated in the usual manner, treated with 200 ml of 4% aqueous permanganate (instead of 300 ml) and maintained at only 60^{o}C in a warm-water bath for a period of 6 hrs. Following the

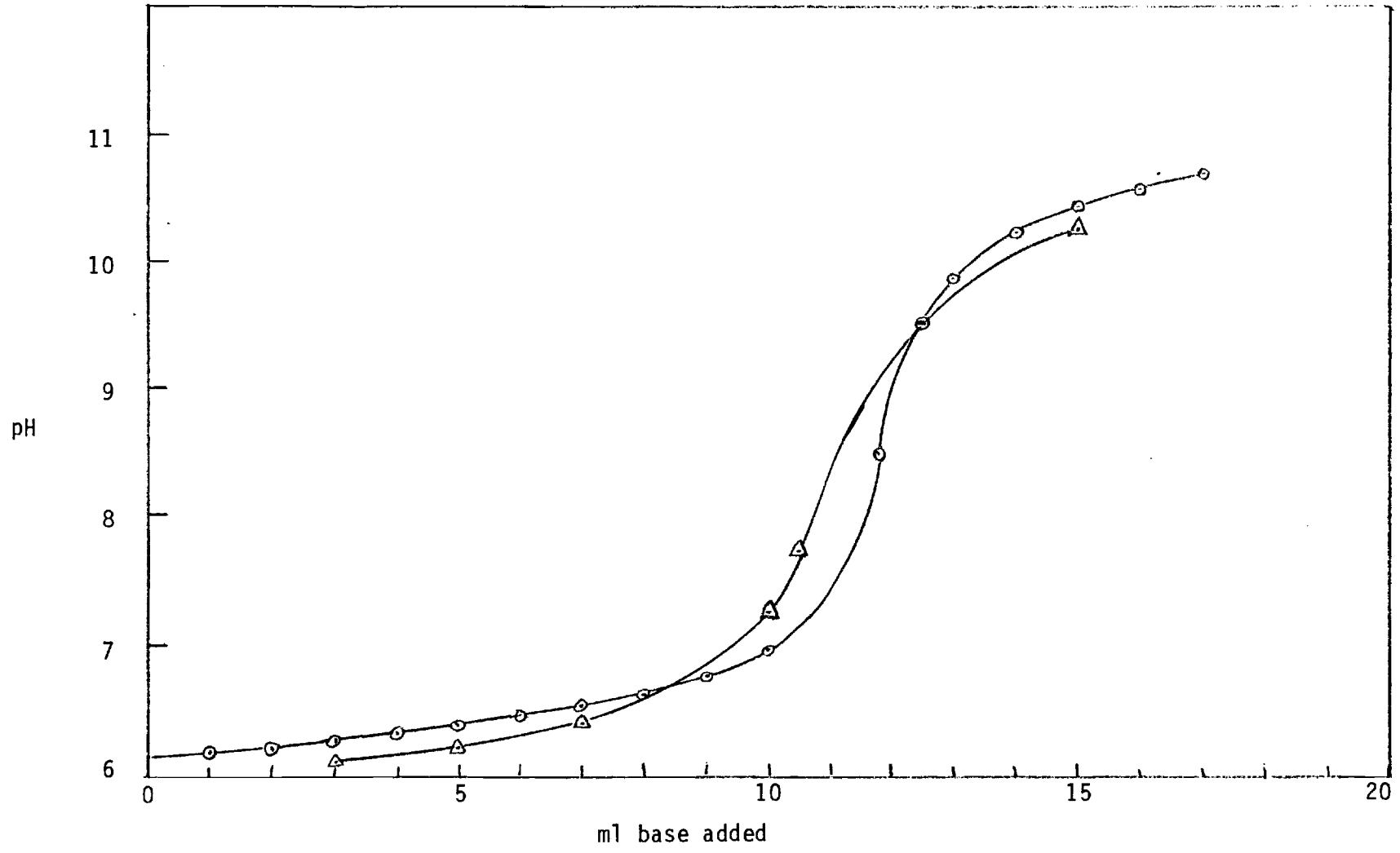

Figure 1. Improved Potentiometric Titration - Humic Acids.

destruction of excess permanganate with methanol, the mixture was steam distilled. This distillate has been subjected to GC/MS analysis.

The residue was filtered to remove MnO_2, concentrated to 50 ml on a rotary evaporator and treated with a large excess of strongly acidic cation exchange resin (Biorad 6-50X8) in order to remove inorganic salts. During the course of this treatment, the pH of the pale yellow solution dropped from strongly alkaline to 3.35. A final pH adjustment to 2.0 was accomplished with hydrochloric acid. The acidified solution was cooled with ice water while being continuously extracted for 10 hours with ethyl acetate in order to minimize transesterification and hydrolysis. Removal of solvent from the dried extracts provided 470 mg of residue. Thus it is apparent that the conversion of the humic matter to carbon dioxide has been greatly reduced. This mixture of products has been methylated and is now being separated and characterized.

Another difficulty with the oxidation of methylated aquatic humics is associated with the fact that they are not soluble in water. Thus they are left behind as a thin film on the surface of the container after removal of the organic solvent used in the methylation reaction. The subsequent oxidation reaction must proceed through this film thus adversely changing the kinetics of the reaction. In order to avoid this condition, water (20 ml) was added to a solution of the methylated humic materials in chloroform and the mixed solvents were then carefully evaporated so that a suspension of methylated humics in water was produced as the organic solvent was removed. The aqueous permanganate was then added in the usual manner.

Extensive GC/MS work on these product mixtures has been performed using both EI and CI methods. These data are presented in the next section.

VI. MASS SPECTRAL ANALYSIS OF OXIDIZED AQUATIC HUMICS

The conventionally oxidized sample M/73 was introduced into the mass spectrometer via a 200' SP 2100 capillary column using He as the carrier gas with split and sweep flows at 29 and 28 ml/min respectively. Temperature programming was employed as follows - hold at 100°C for 4 minutes; increase at 1.5°/min to 130°C; then 3°/min to 220°C and hold at 220°C. Methane was used as the make up and reactant gas for chemical ionization. In this way it was possible to overlay the EI and CI chromatograms even in an expanded scale mode. Thus the information gained from each of the techniques is fully complimentary. Figures 2 and 3 illustrate the overlap for the first two hundred scans. Figures 4 and 5 illustrate the complementary nature of the second two hundred scans. This pattern continues with only a slight offsetting thoughout the useful portions of the total ion chromatograms. Relative peak heights are not the same due to differences in ionization efficiencies coupled with the generally lower sensitivities shown by the CI method. The relatively cleaner ion maps seen in the CI spectra should also be noted. The complete total ion chromatograms are presented in Figures 6 and 7. These figures can be used to estimate the relative proportions and locations in the elution patterns of each of the compounds identified in Table I.

Of the compounds identified in Table I, several have been selected for presentation on the following pages, either because their structures have been assigned on the basis of interpretation rather than on the basis of a "hit" with library spectra, or because an interesting fragmentation pattern is exhibited. For example, Figures 12 and 13 are to be compared so that the

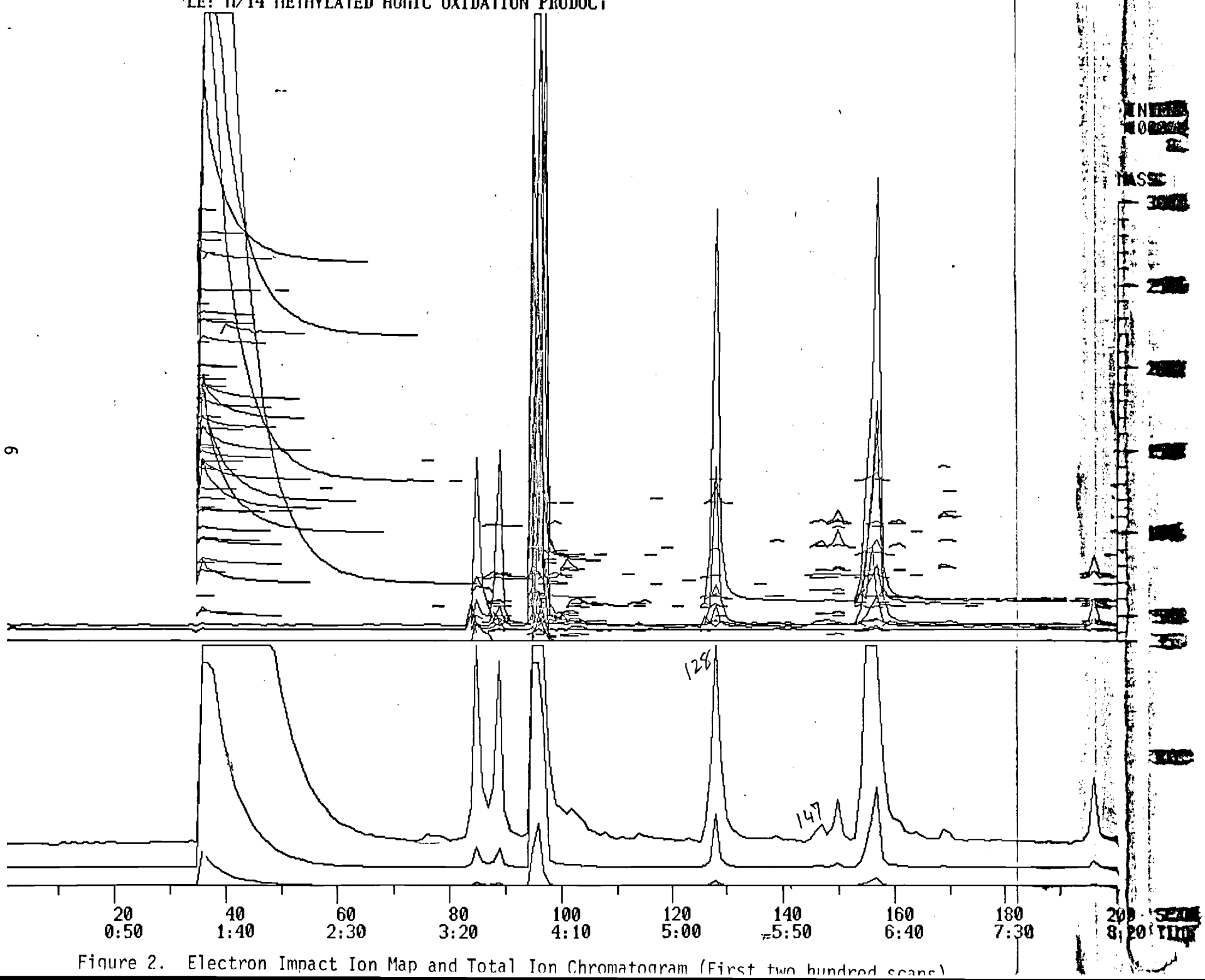

Figure 2. Electron Impact Ion Map and Total Ion Chromatogram (First two hundred scans)

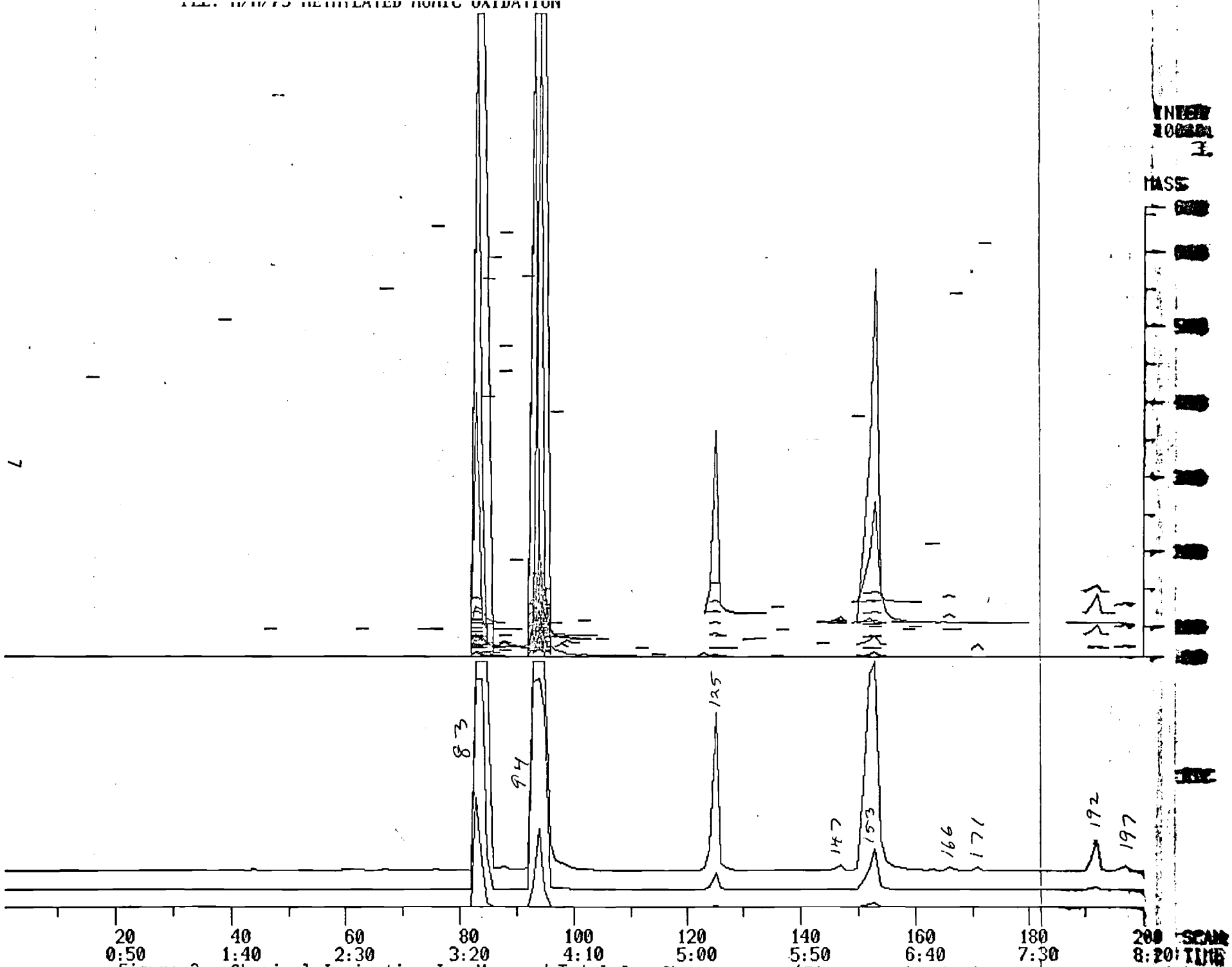

Figure 3. Chemical Ionization Ion Map and Total Ion Chromatogram. (First two hundred scans - compare with Fig. 2).

SAMPLE: M/14 METHYLATED HUMIC OXIDATION PRODUCT

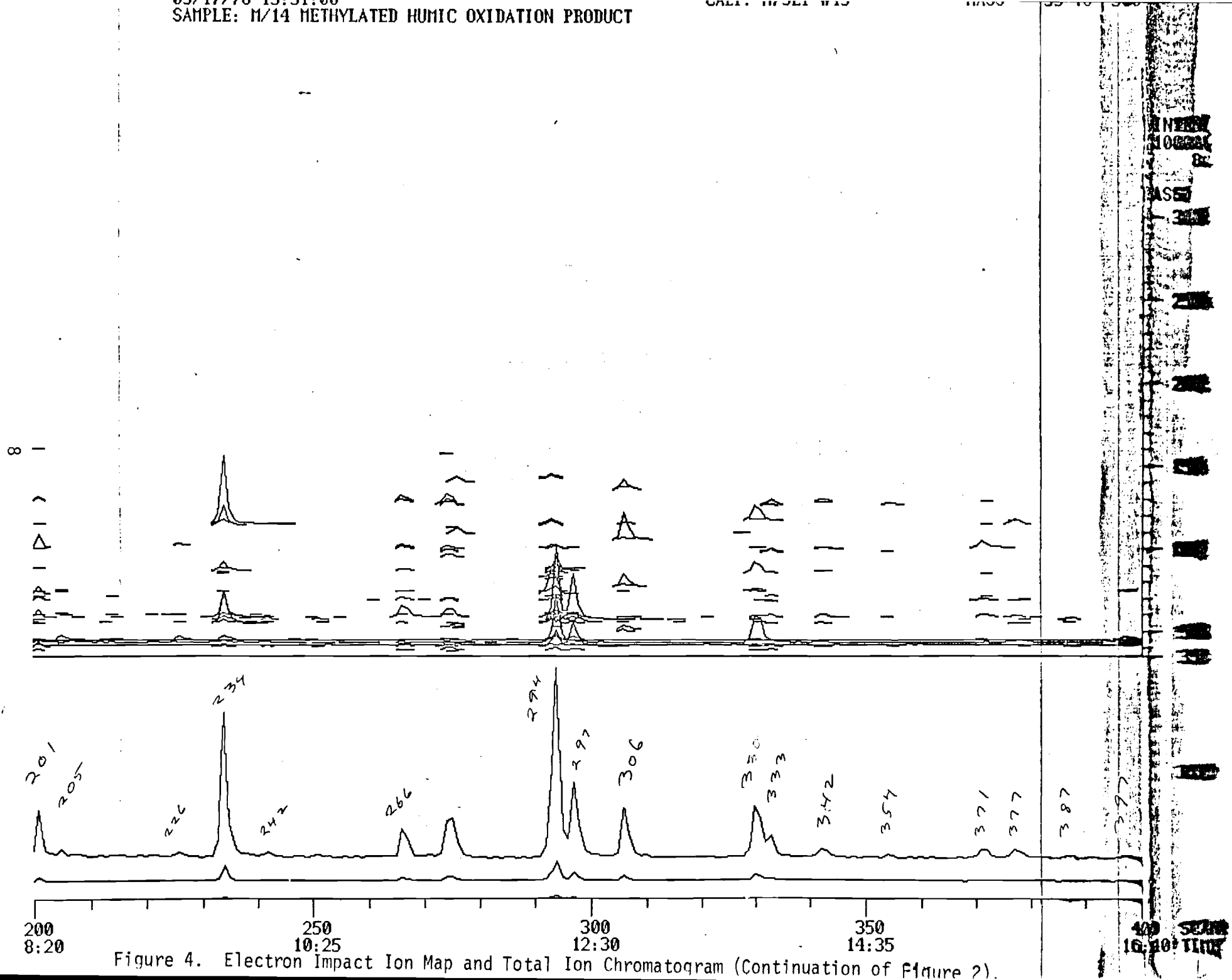

Figure 4. Electron Impact Ion Map and Total Ion Chromatogram (Continuation of Figure 2).

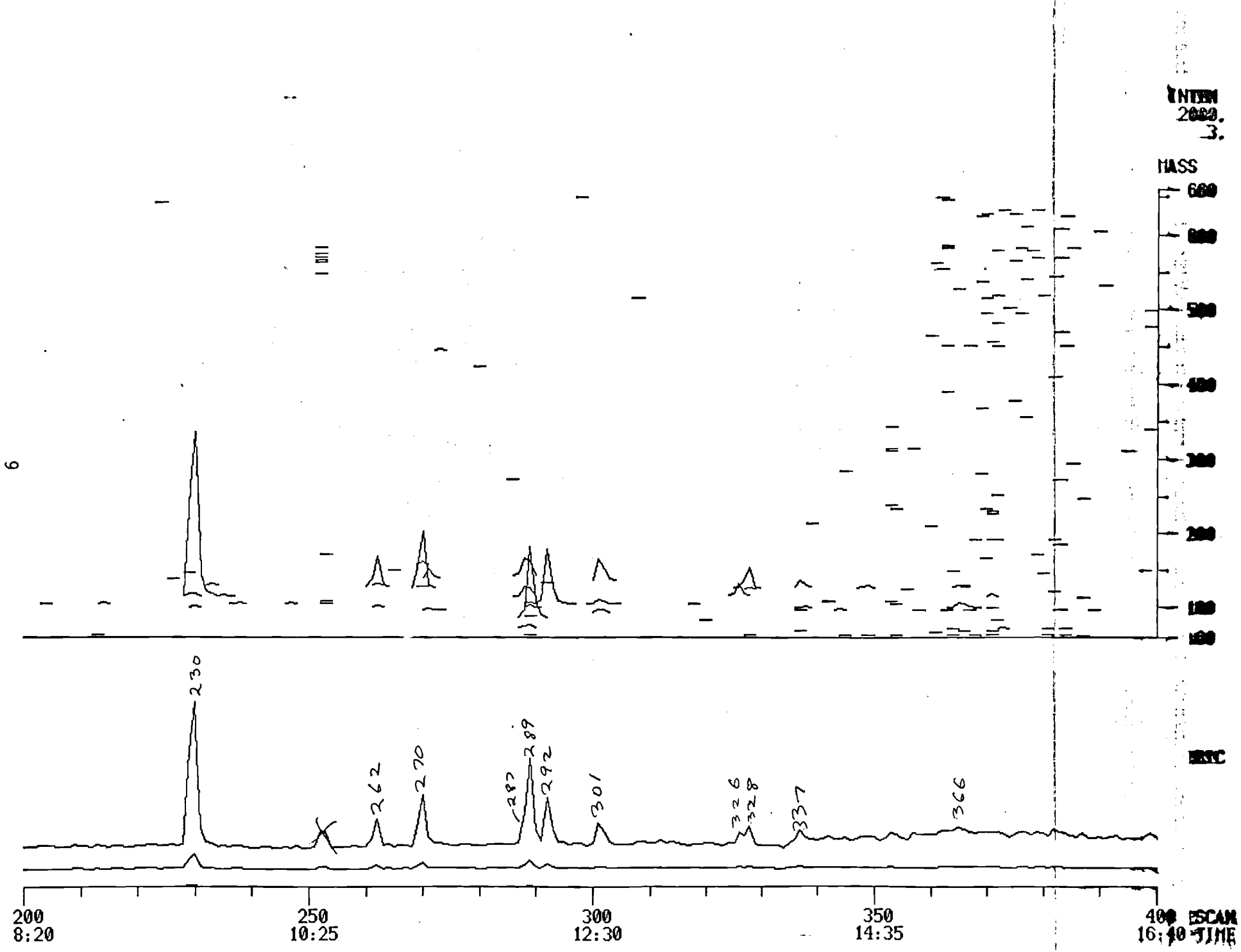

Figure 5. Chemical Ionization Ion Chromatogram (Continuation of Figure 3 - compare with Figure 4.)

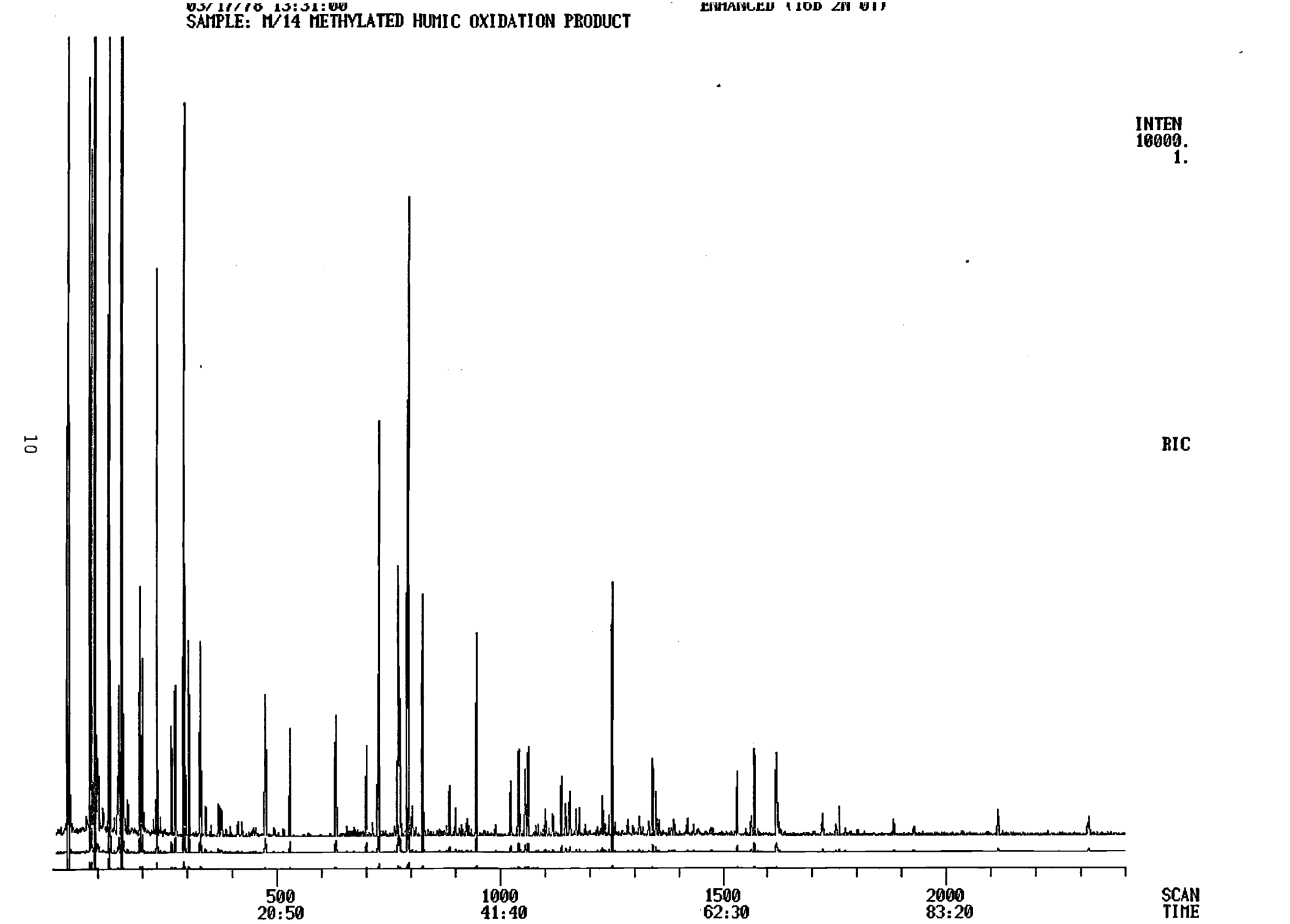

Figure 6. EI - Ion Chromatogram Methylated Humic Oxidation Products

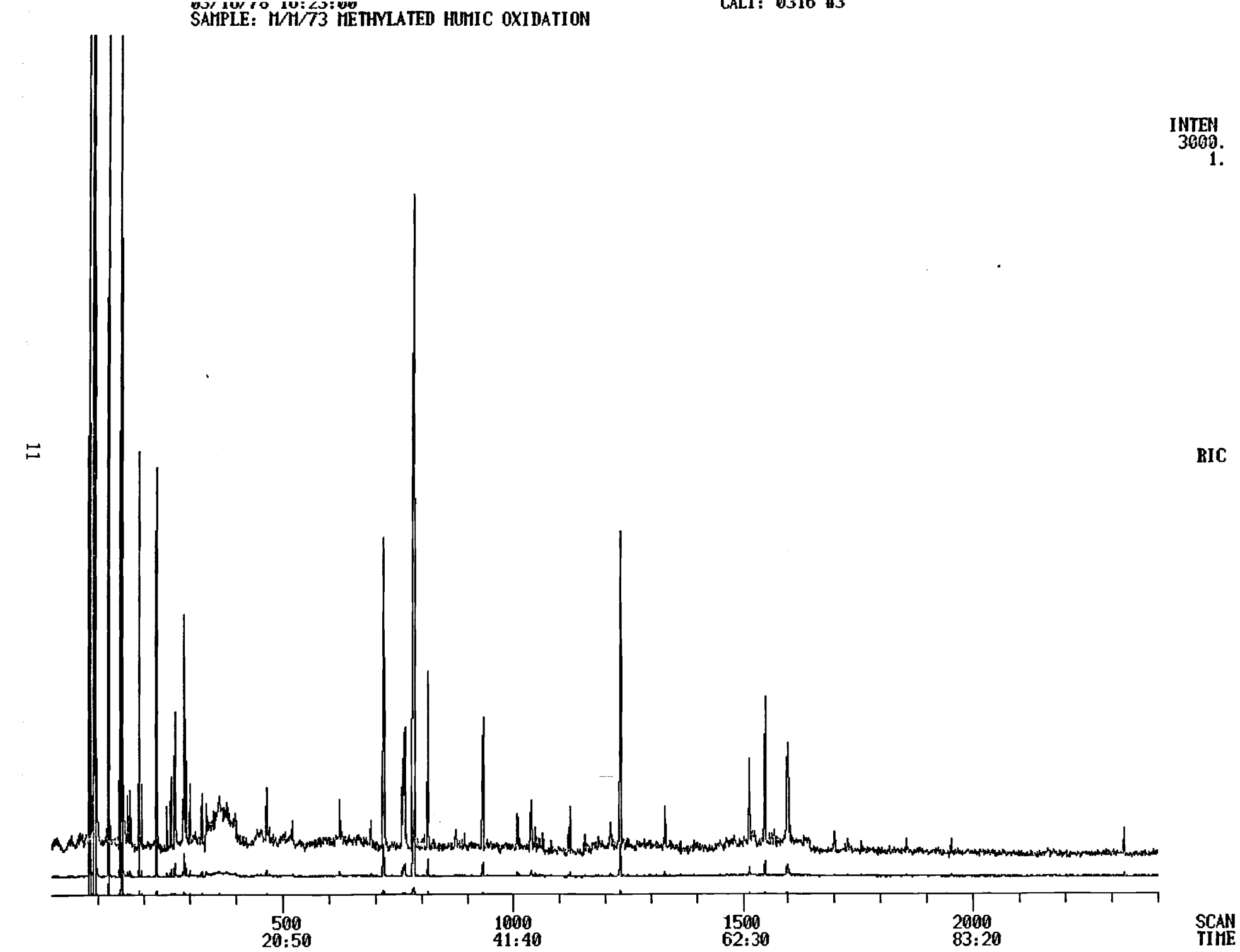

Figure 7. CI Ion Chromatogram Methylated Humic Oxidation Products

TABLE I

Mass Spectral Data of Methylated Humic Oxidation Products

Comment	Chemical Ionization Scan No.	Chemical Ionization Major Peak(s)[a]	Comment	Assigned Structure	Figure(s)
M^+, CO_2Me	125	119	M+1	Dimethyl Oxalate	not included
loss of OMe, split at CO_2Me	229	147,115	M+1, loss of OMe	Dimethyl Succinate	not included
loss of OMe, CO_2Me, CH_2CO_2Me; CO_2Me^+	262	129,101	loss of OMe, CO_2Me	Methyl Succinic Acid Biester	not included
M^+; loss of Me, OMe, CO_2Me, CO_2Me+Me	NA			5-MethylFuran-2-carboxylic acid ester	Figure 8
M^+, loss of OMe, CO_2Me	301	137,105	M+1, loss of OMe	Methyl Benzoate	not included
loss of OMe, MeOH, CO_2Me, CO_2Me+H, CH_2CO_2Me; McLafferty, CO_2Me^+	337	129,101	loss of OMe, CO_2Me	Dimethyl Glutarate	Figure 9
loss of OMe, CO_2Me, ?; $CH_2CO_2Me^+$, CO_2Me^+	NA			Dimethyl Dimethyl Succinate	Figure 10
M^+, loss of OMe; $C_6H_5^+$	NA			Methyl p-Methoxy Benzoate	not included
M^+, loss of OMe, CO_2Me, OMe+CO_2Me, ?; $C_6H_5^+$	815	163	loss of OMe	Dimethyl Phthalate	not included
M^+,?,loss of OMe,?, CO_2Me,OMe+CO_2Me; $C_6H_5^+$	875	195,151,147, 118	M+1,?	Dimethyl Isophthalate	not included
M^+,loss of OMe, CO_2Me,MeOH+CO_2Me; 2 CO_2Me's	890	195,97,72	M+1,?	Dimethyl Terephthalate	not included

Comment	Scan No.	Chemical Ionization Major Peak(s)[a]	Comment	Assigned Structure	Figure(s)
loss of OMe	NA	CI data beyond this point is still being processed		Benzene 1,2,3 tricarboxylate	not included (see previous report)
M^+, loss of OMe, CO_2Me, OMe+CO_2Me	NA			Benzene 1,3,5 tricarboxylate	
M^+, $(CH_2)_6CO_2Me$, $(CH_2)_2CO_2Me$ McLafferty				14-Methyl pentadecanoate	not included
				Benzene 1,2,4 tricarboxylate with some hydrocarbon background?	Figure 11
M^+, loss of OMe, CO_2Me, CH_2O by rearrangement+ 2 CO_2Me				Benzene 1,2,4,5 tetracarboxylate (because M^+ is seen)	Figure 12
loss of OMe, CH_2O by rearrangement+ 2 CO_2Me, CH_2O+3 CO_2Me				Benzene tetracarboxylate	Figure 13
loss of OMe, 162 as before; compares with authentic sample				Benzene pentacarboxylate	not included

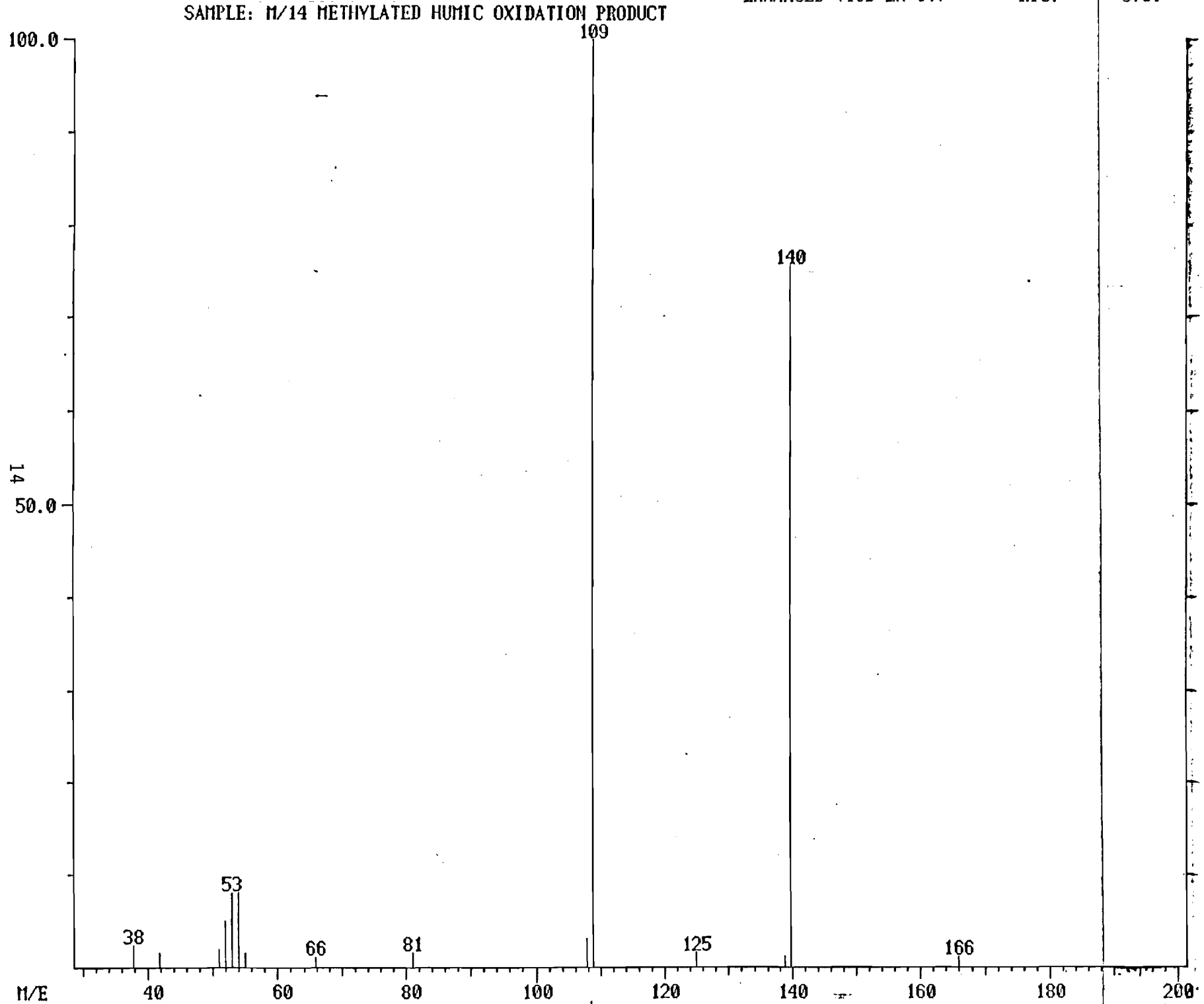

Figure 8. Electron Impact Spectrum 5-Methylfuran-2-Carboxylic Acid Methyl Ester (has been reported earlier)

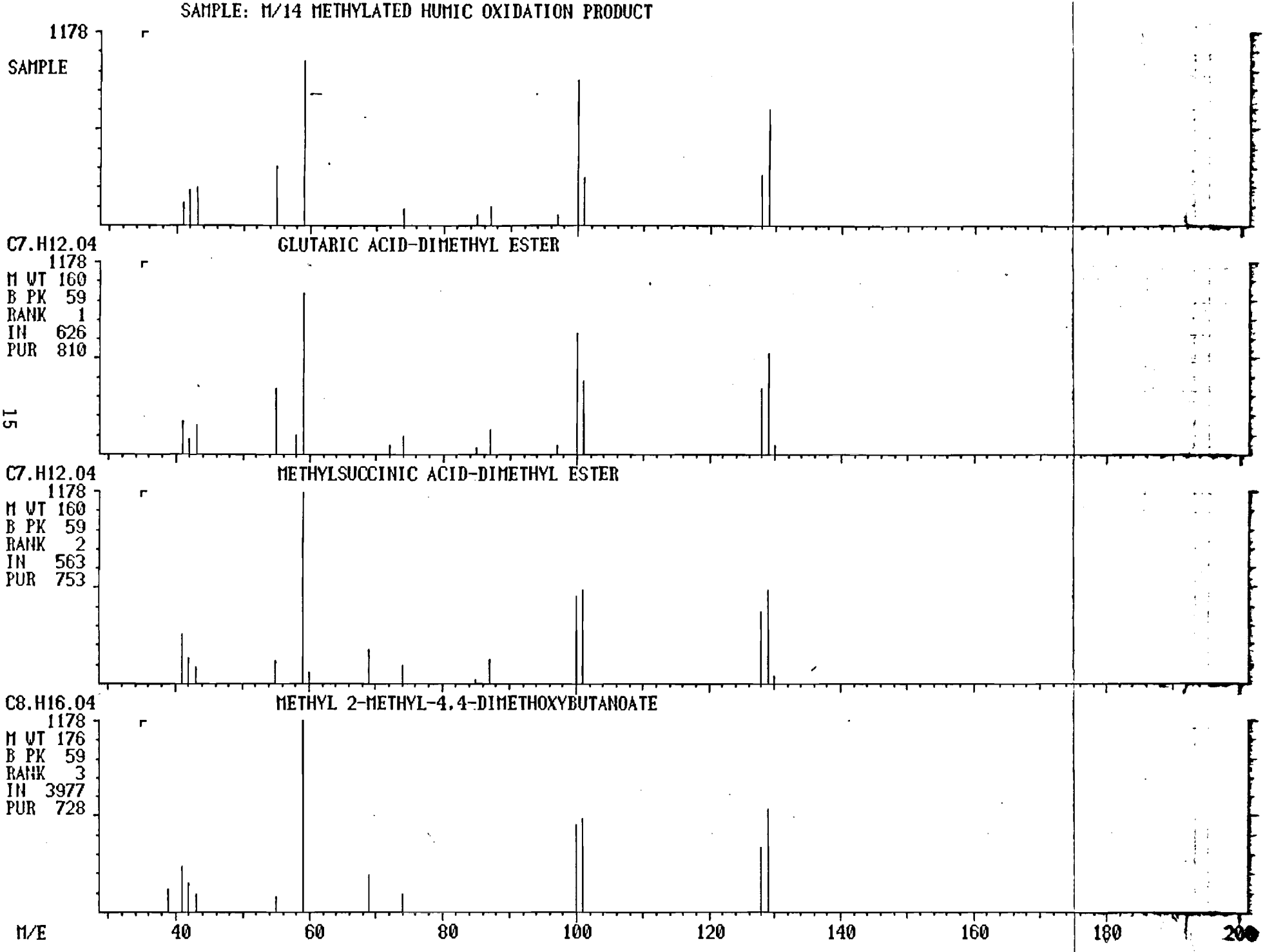

Figure 9. Electron Impact Spectrum Peak 342 (top). Methylated Humic Oxidation Products Showing Comparison with Dimethyl Glutarate and other Choices.

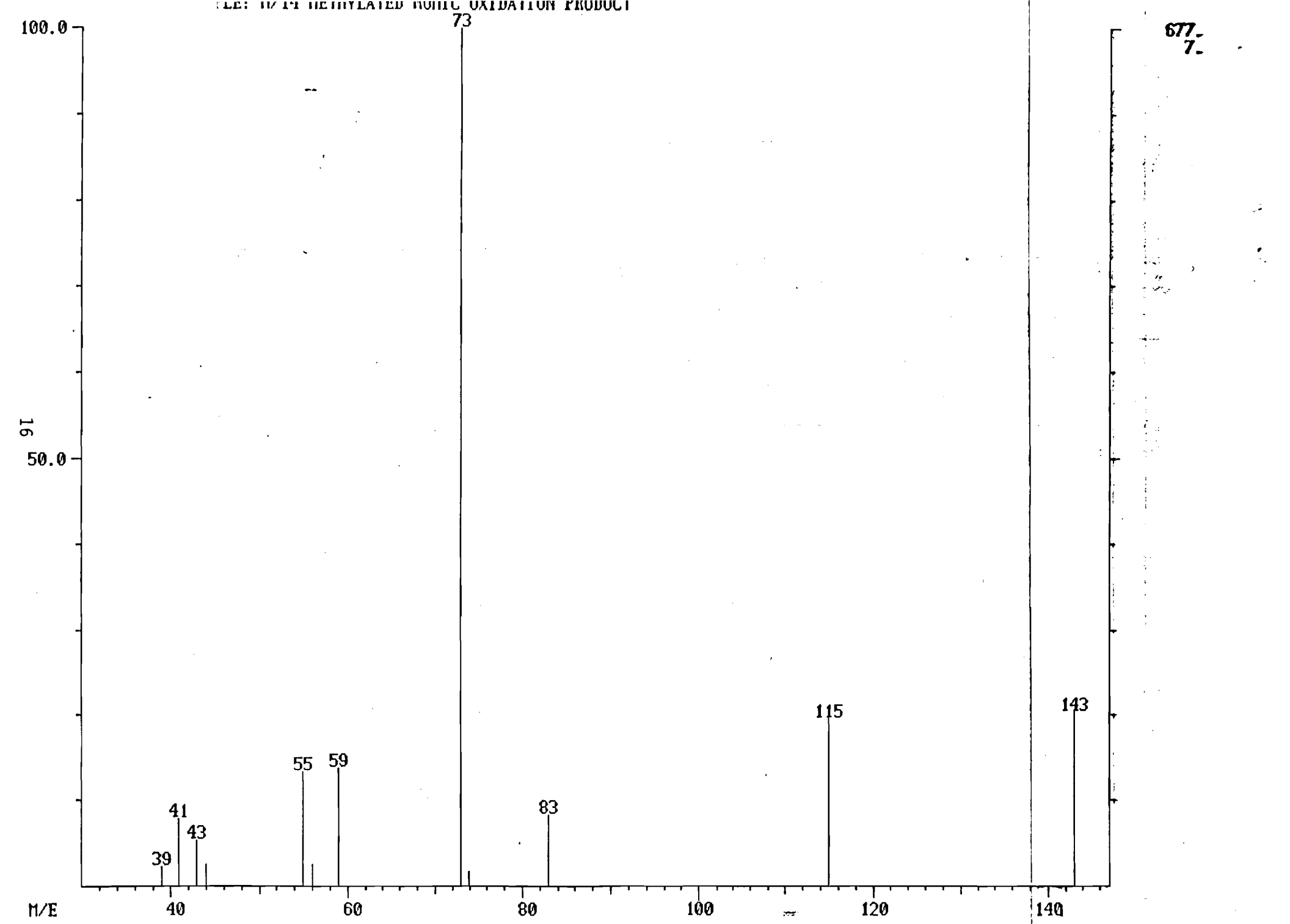

Figure 10. Dimethyl Dimethyl Succinate. Electron Impact Spectrum Peak 531 - Methylated Humic Oxidation Products

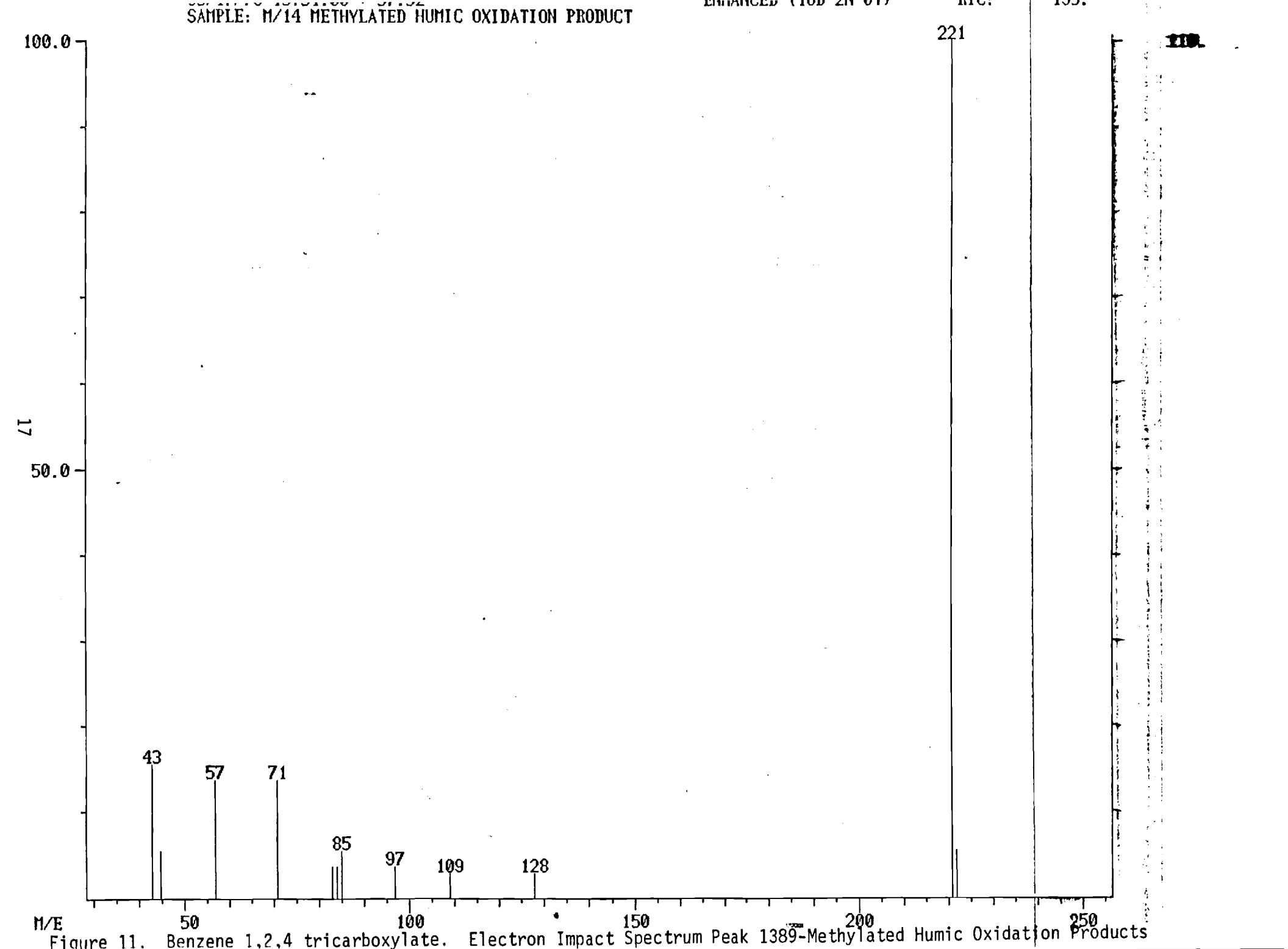

Figure 11. Benzene 1,2,4 tricarboxylate. Electron Impact Spectrum Peak 1389-Methylated Humic Oxidation Products

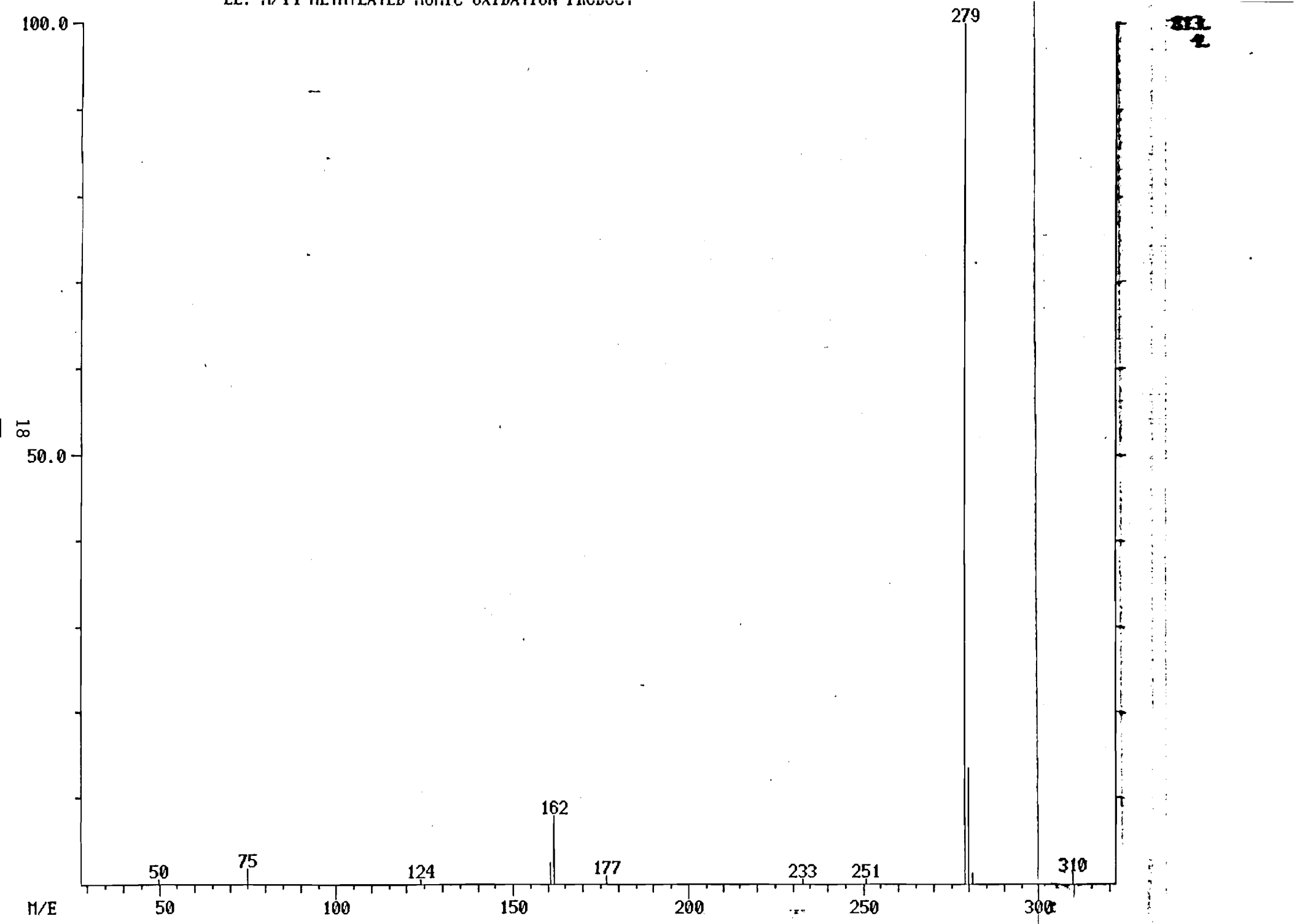

Figure 12. Benzene 1,2,4,5-Tetracarboxylate. Electron Impact Spectrum Peak 1574-Methylated Humic Oxidation Products

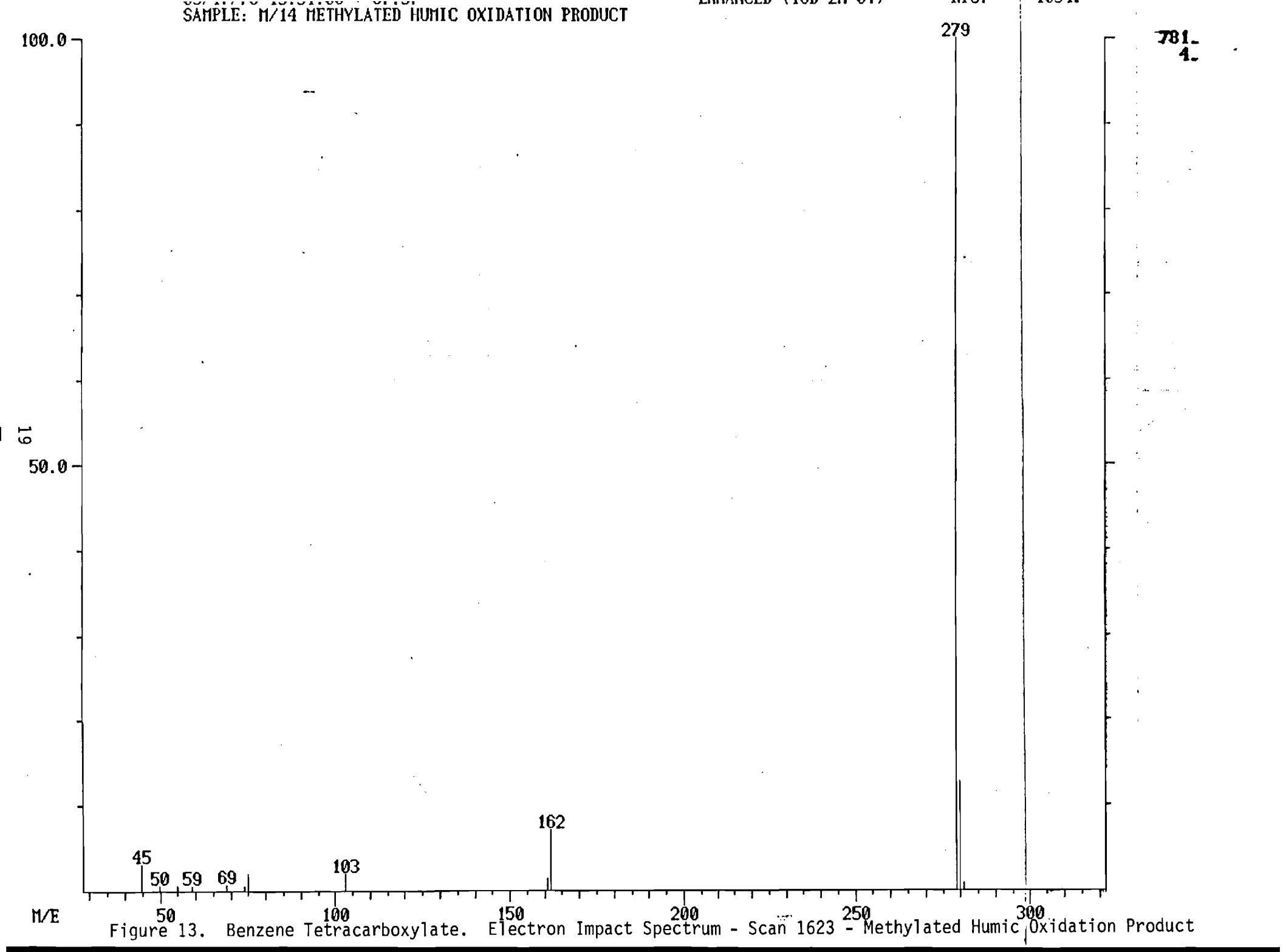

Figure 13. Benzene Tetracarboxylate. Electron Impact Spectrum - Scan 1623 - Methylated Humic Oxidation Product

rationale for assigning the 1,2,4,5 structure to one of the benzene tetracarboxylates (Figure 12) can be more readily understood. All of the included spectra provide evidence to support the observation that loss of methoxyl and methyl carboxylate are highly favored processes. A very large quantity of data from the other oxidation experiments is now being examined.

VII. JAR TESTS TO DEVELOP CONDITIONS FOR FLOCCULATION DURING DISINFECTION OF AQUATIC HUMICS IN "MINI-PLANT" OPERATION

The objective of the first series of jar tests was to find conditions which would produce a settleable flocculation by adding reagents to an aquatic humic solution. The solutions under test contained pH adjustment compounds which would serve to maintain the pH between 6-8 in the mixing chamber of the mini-plant. This step is necessary because the aqueous chlorine solution which is added during the water treatment simulation has a low pH. It was recognized that commercial practice for removal of humic material from drinking water supplies generally employs a pH in the range of 5 to 6.5 during flocculation. While it may not be possible to rigorously control the pH over a narrow range and still accomplish flocculation of aquatic humic solutions, a search for conditions which might accomplish this goal was considered to be important. The method used for the standard jar tests is that which is described in Standard Methods.

A 3L capacity cylindrical battery jar was used for a series of tests. The contents of the jar were mixed by manual swirling. Observations were made on the unstirred contents of the jar over a period of at least 15 minutes. The concentration of the aquatic humic material (fraction M/30) ranged from 10 mg/liter to 10mg/1.1 L. The temperature was in the range of 22-24^{o} during the tests.

The composition of the test solutions or suspensions, the pH of the aquatic humic solution containing buffer components and the pH after all components of the jar test mixtures had been added are summarized in Table II. The results of runs 1 through 15 can be summarized by stating that various degrees of turbidity were produced with no floc formation and no settling of particulate material (with the exception of the larger clay particles) during the fifteen minute test period.

Run 16 represents the only set of conditions which produced a floc within five minutes of mixing. This floc was quite well settled within 10 minutes of mixing. The measurement of the height of the sludge-liquid interface for three times after resuspension gave the values shown in Table III.

A plot of these data is shown in Figure 14. The slope of the best line drawn through the experimental points was 0.23 inches per minute. This value gives a zone settling rate of 1.2 feet per hour.

The use of 100 mg of ferric chloride and 500 mg of aluminum sulfate for each 10 mg of aquatic humic material in a liter of solution is a very high dosage which would seem to have little relationship to large scale water treatment practices.

VIII. CONCERNING THE ACID GENERATION OF CHLORINE FROM HYPOCHLORITE

A series of experiments was undertaken to evaluate the possibility that the sulfuric acid used to liberate free chlorine from prepurged commercial hypochlorite might also be generating excess hydrochloric acid from chlorides in the commercial solution. In the first experiment of this series, the trap was filled with high purity water and after 15 minutes the chlorine solution was replaced with a fresh charge of high purity water and the generation was

TABLE II

Jar Test Conditions for Flocculation of Aquatic Humic Solution

Run No.	K_2HPO_4 mg	KH_2PO_4 mg	$NaHCO_3$ mg	Na_2CO_3 mg	Vol. L	pH M/30, Buffer	$FeCl_3 6H_2O$,mg	$Al_2(SO_4)_3 nH_2O$,mg	Clay, mg	Final Vol.L	Final pH
1	1000	300	0	0	1.0	7.28	100	0	0	1.05	7.21
2	1000	300	0	0	1.0	7.25	0	100	0	1.05	6.92
3	1000	300	0	0	1.0	7.26		100	100	1.05	7.01
4	1000	300	0	0	1.0	7.28	0	0	100	1.0	7.28
5	0	0	667	0	1.0	8.32	100	0	0	1.05	8.19
6	0	0	667	0	1.0	8.33	0	100	0	1.05	7.99
7	0	0	667	0	1.0	8.31	0	100	100	1.05	7.97
8	0	0	0	750	1.0	10.28	100	0	0	1.05	9.89
9	0	0	0	750	1.0	10.30	100	0	100	1.05	9.99
10	0	0	0	750	1.0	10.26	200	0	100	1.10	9.92
11	0	0	0	750	1.0	10.22	0	100	0	1.05	9.85
12	0	0	0	750	1.0	10.24	0	100	100	1.05	9.62
13	0	0	0	750	1.05	9.67	0	200	100	1.10	9.22
14	0	0	0	750	1.05	10.27	100	20	0	1.07	9.34
15	0	0	0	700	1.0	9.94	0	500	0	1.0	8.12
16	0	0	0	700	1.0	9.94	100	500	0	1.0	7.13

TABLE III

Height of Sludge-Liquid Interface
for a Treated Aquatic Humic Solution

Time, Minutes	Height of Interface, Inches		
	1	2	3
1	2.38	2.25	2.13
2	2.0	2.0	1.87
3	1.5	1.63	1.75
5	1.0	1.13	1.13
10	0.25	0.125	0.25

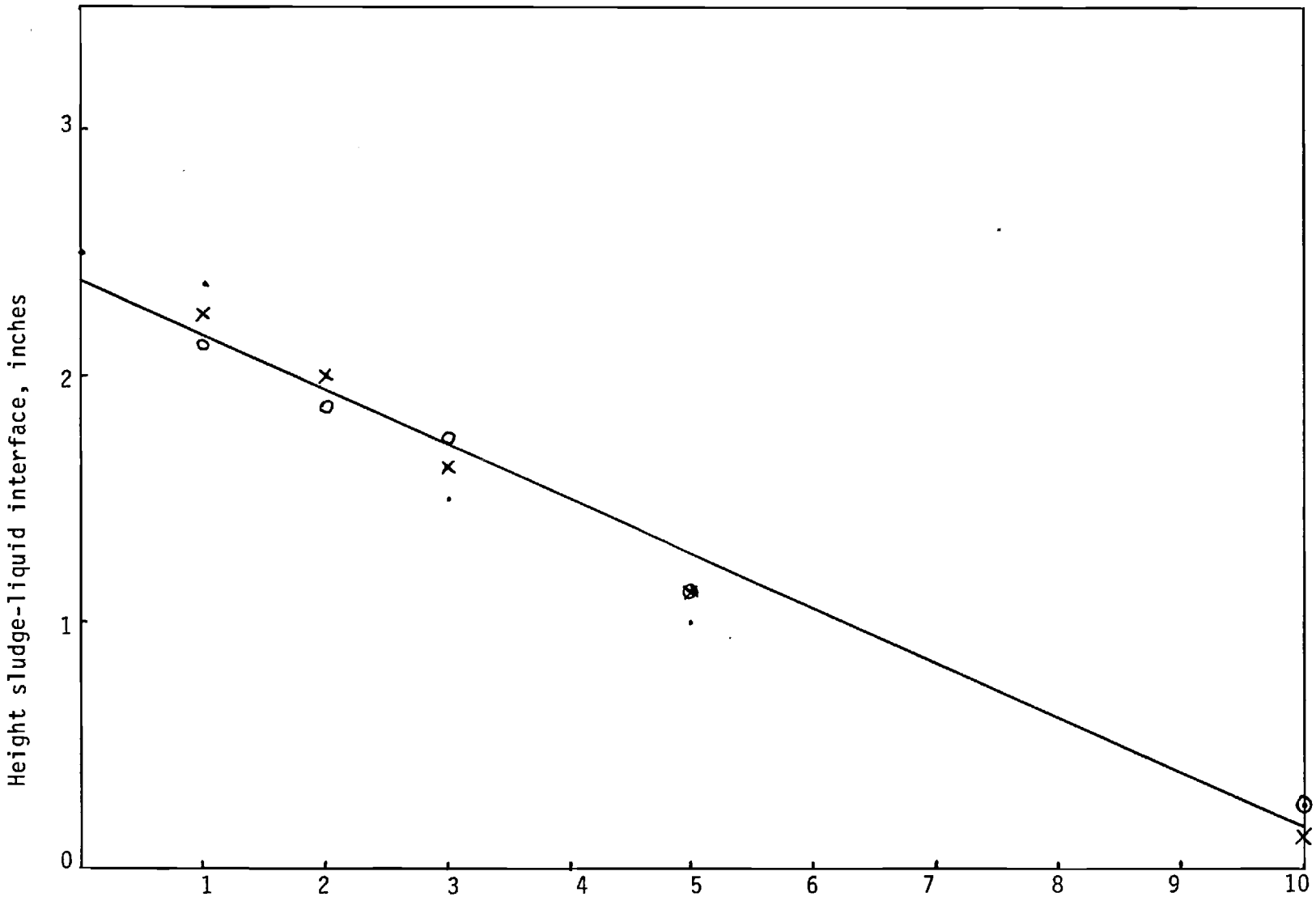

Figure 14. Floc Settling of Buffered Aquatic Humic Material Treated with Fabric Chloride and Aluminum Sulfate.

continued an additional 30 minutes. The chlorine solutions produced in this manner were found to contain 3.6 mg/ml and 1.4 mg/ml free chlorine, respectively. The observed pH values were 1.98 and 2.32. If significant carryover of hydrochloric acid had occurred, the second, longer run out of the same generator flask should have had the lower pH. Instead, the pH seemed to be related to the following equilibrium:

$$Cl_2 \rightleftharpoons HOCl + HCl$$

The final experiment in this series employed 4% sodium chloride solution in the generator in place of the commercial hypochlorite. The sulfuric acid addition, nitrogen purging and trapping were carried out as before. After 30 minutes, the pH of the water in the trap rose from 5.52 to 6.12. The increase in pH in the trapping water was probably due to removal of carbon dioxide by the nitrogen gas stream. These observations clearly indicate that no hydrogen chloride is transferred into the chlorine solution under the conditions used for the sodium hypochlorite-dilute sulfuric acid reaction.

IX. REACTION OF AQUATIC HUMIC MATERIAL WITH CHLORINE

A sample of aquatic humic material from the Satilla River weighing 100 mg was dissolved in 10 liters of high purity water containing 10 g K_2HPO_4 and 3 g KH_2PO_4. The humic matter was first added to a solution of the dipotassium hydrogen phosphate so that the lower pH could speed up the dissolution process. The potassium dihydrogen phosphate was then added to establish the proper pH of 7.3. The resulting solution was subsequently brought to volume. The flow rate of this solution from the reservoir was adjusted to 1.0 liters per hour.

A solution of chlorine in high purity water was prepared in the usual way and added at such a rate (50 ml/hr) as to maintain a residual throughout

the system. The strength of this solution was measured by the DPD ferrous titrimetric method* and was found to be 3.6 mg/ml. Thus on a weight/weight basis, an 18 fold excess of chlorine was present. The pH in the mixing chamber was found to be 7.2 with a chlorine residual of 76 mg/l. The pH of the eluate from the sand filter was 7.03. The chlorine residual at this point was 20 mg/l. The pH of the liquid in the settling chamber was 7.13. After standing overnight, the final chlorine residual had dropped to 1.8 mg/l in the settling chamber. Samples were taken from the reservoir, the sand filter eluate and the treated water which had stood in the settling chamber overnight. These are in various stages of workup at present. It should be noted that this run was carried out without the addition of a flocculating agent. A companion run will be carried out with a flocculating agent in the immediate future.

The effluent from the sand filter which would correspond to the finished water in many full-scale plants was treated with excess sodium sulfite to quench the residual chlorine. This solution was adjusted to pH 1.5 with sulfuric acid and extracted with distilled-in-glass ethyl ether (3×200 ml). The ether extracts were then dried over roasted sodium sulfate prior to concentration to 6 ml on a freshly cleaned Kuderna-Danish apparatus. This concentrate was treated with diazoethane in the usual manner during which time the evolution of nitrogen was observed. The reaction mixture containing the ethylated post-treatment humic acid oxidation/chlorination reaction products was concentrated to 3.5 ml. A 1-liter portion of the unreacted aquatic humic material remaining in the mini-pilot reservoir was extracted, worked up and concentrated in a similar manner.

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*Standard methods, 14th edition, page 329 (1975).

The aforementioned reacted material has been analyzed by capilary column GC/MS using the techniques of both EI and CI mass spectrometry. The mass spectra of many of the components of this complex mixture (see Figure 15) exhibit the characteristic isotope ratios expected for chlorinated materials. On this basis, 40-50% of the chromatographic peaks shown in Figure 15 can be said to contain chlorine. By contrast, the EI spectra derived from the control (see Figure 16) for the total ion chromatogram do not exhibit this characteristic isotope ratio distribution. The fact that several components are seen in this material is also a matter of interest. It may be that standing overnight in air coupled with the conditions of extraction (acid pH) are sufficient to bring about a partial degradation of aquatic humic matter. Thus, this technique in itself may provide further information regarding the structure of such materials.

Since this experiment was run towards the end of the reporting period, there has not yet been time for more than a preliminary interpretation of these exciting results. Since the GC/MS library would not be expected to contain a large proportion of chlorinated compounds, most of our conclusions will have to be based on our own interpretation of the data. Some of the interpreted data have been summarized in Table IV.

The complimentary chemical ionization data plus both the electron impact and chemical ionization data from the control together with the remaining EI data from the chlorinated aquatic humics are now being processed by our staff. It can be expected that more reaction products will be identified during the next reporting period. Needless to say, the proportion of chlorinated organics in the reaction mixture is highly signifcant. It is our intention to pursue this aspect of the work very actively.

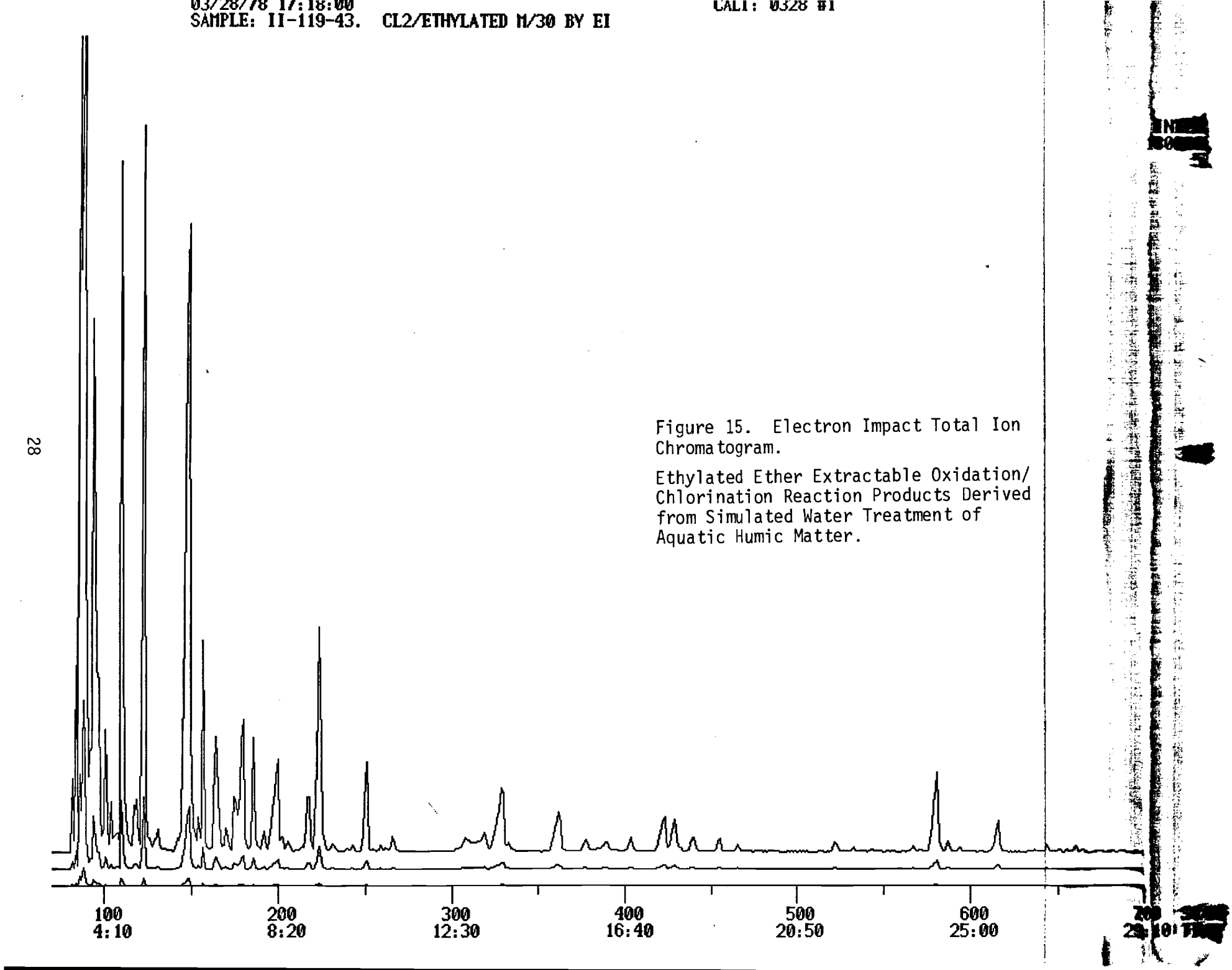


Figure 15. Electron Impact Total Ion Chromatogram.

Ethylated Ether Extractable Oxidation/ Chlorination Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter.

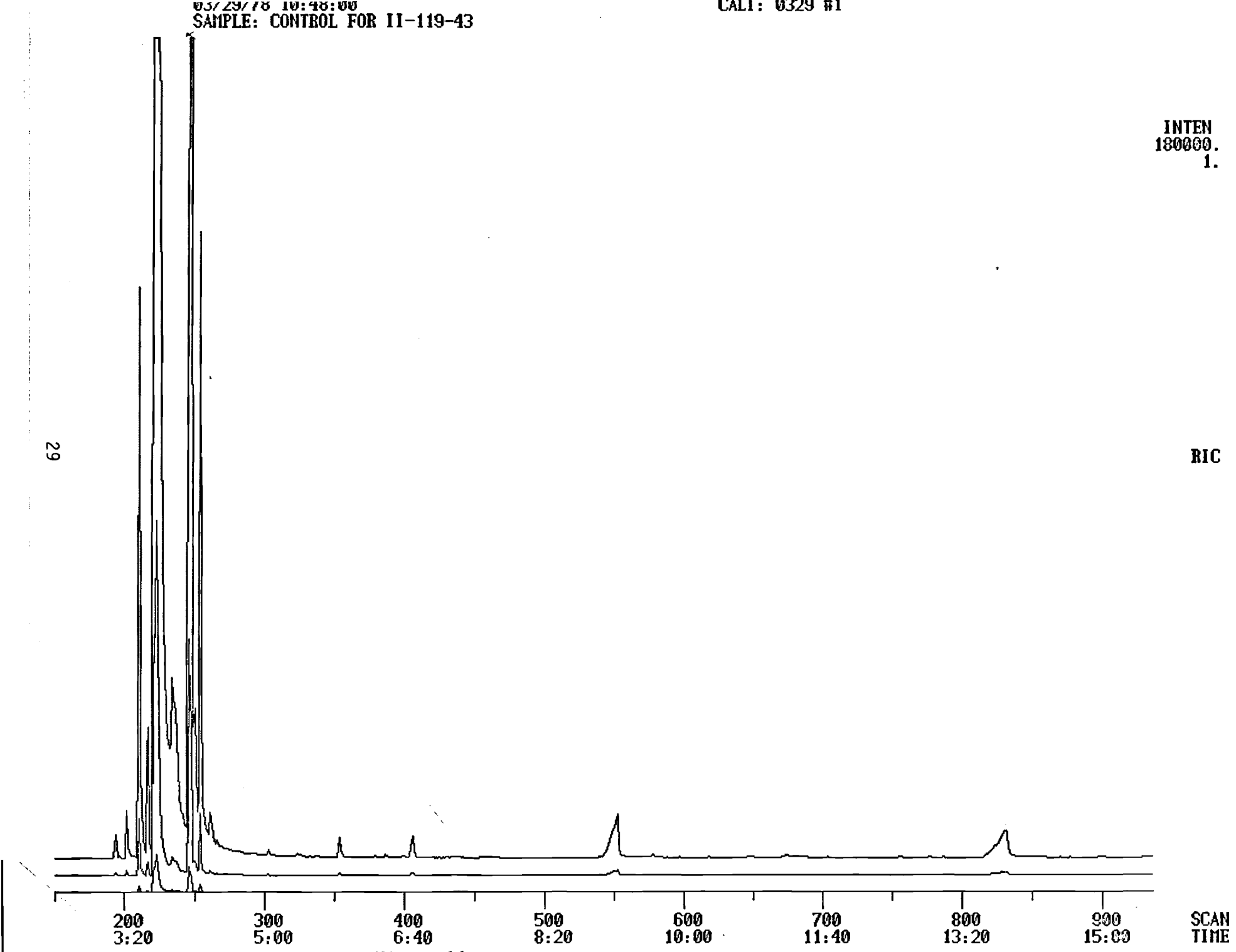


Figure 16. EI Ion Chromatogram - Ethylated Humic Control.

TABLE IV

Mass Spectral Data - Aquatic Humics Reaction Products
Following Simulated Water Treatment

Scan No.	Major Ions	Interpretation	Structure	Figure
110	104,89,69,53, 41	M^+,loss of Me,Cl, Me+HCl,C_2H_4Cl	2-chloro-3-methyl butene-2 or isomer	17
117	103,73,59,45	loss of Me, CH_2OEt, CH_2CH_2OEt	ethylene glycol diethyl ether (probable artifact)	18
119	104,89,69,59, 55,53,41	M^+,loss of Me, Cl; CH_2Cl^+,MeC= $CHMe^+$,Me+HCl, C_2H_4Cl	1-chloro-2-methyl butene-2 or isomer	19
123	100,71,57,43	M^+,loss of Et, Pr; $C_3H_7^+$	3-hexanone or 2-methyl-3-pentanone	20
157	125,105,89,77, 63,41	loss of Me,Cl, HCl+Me; Me_2CCl^+; loss of C_2H_4+HCl; $MeCHCl^+$,$C_3H_5^+$	2,3-dichloro-2-methylbutane	21
163	107,93,73,57, 43	$C_3H_6NOCl^+$,$C_2H_4NOCl^+$, $C_2H_5NO^+$,CH_2Cl^+, $CONH^+$	chlorinated nitrogen compound	22
333	155,117,111,110, 93,83,82,73	loss of Cl,CO_2Et; $CHOCl_2^+$;loss of HCl+CH_3CHO,?; $CHCl_2^+$,CCl_2^+, CO_2Et^+	ethyl trichoro-acetate	23

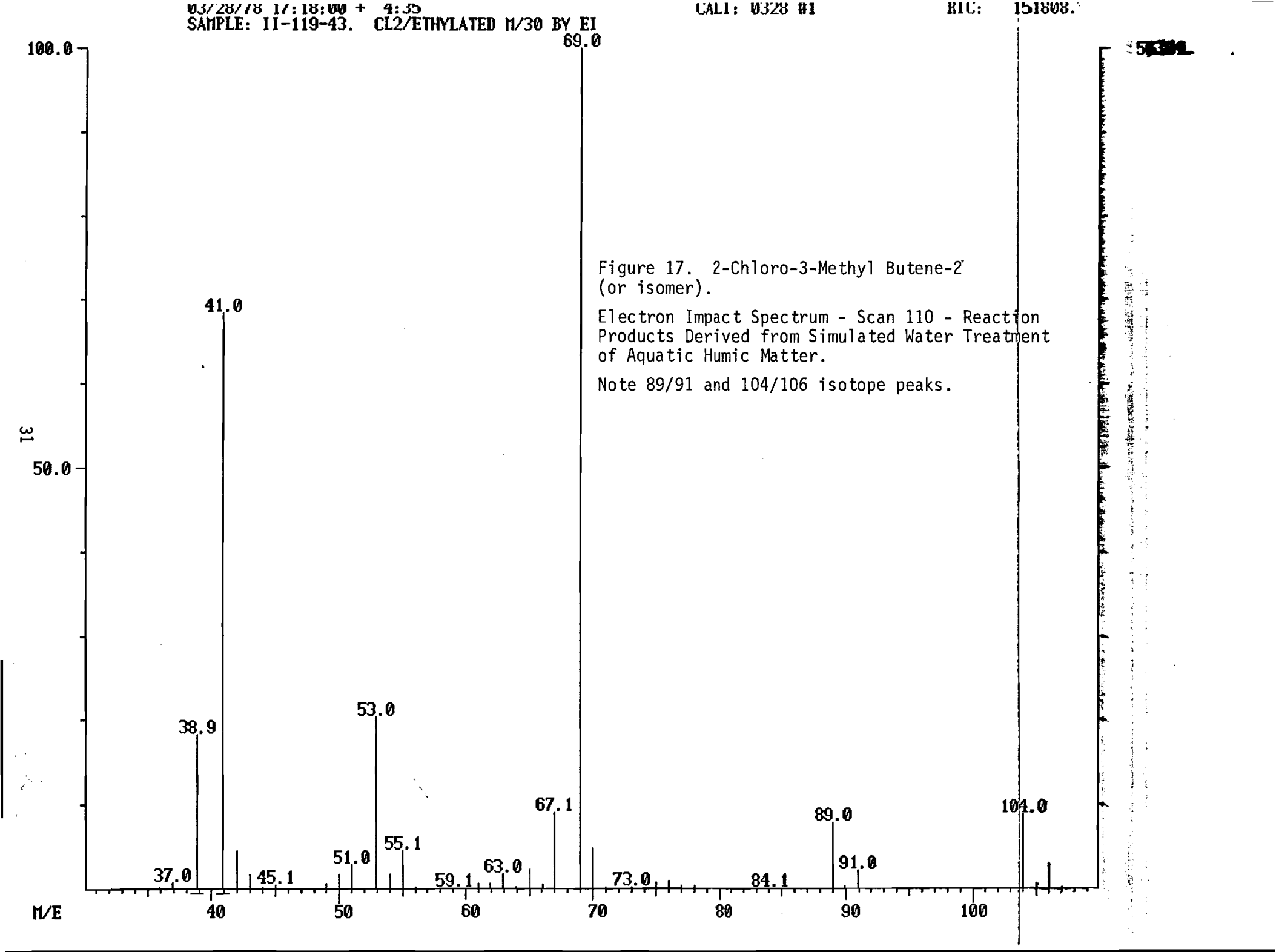


Figure 17. 2-Chloro-3-Methyl Butene-2 (or isomer).

Electron Impact Spectrum - Scan 110 - Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter.

Note 89/91 and 104/106 isotope peaks.

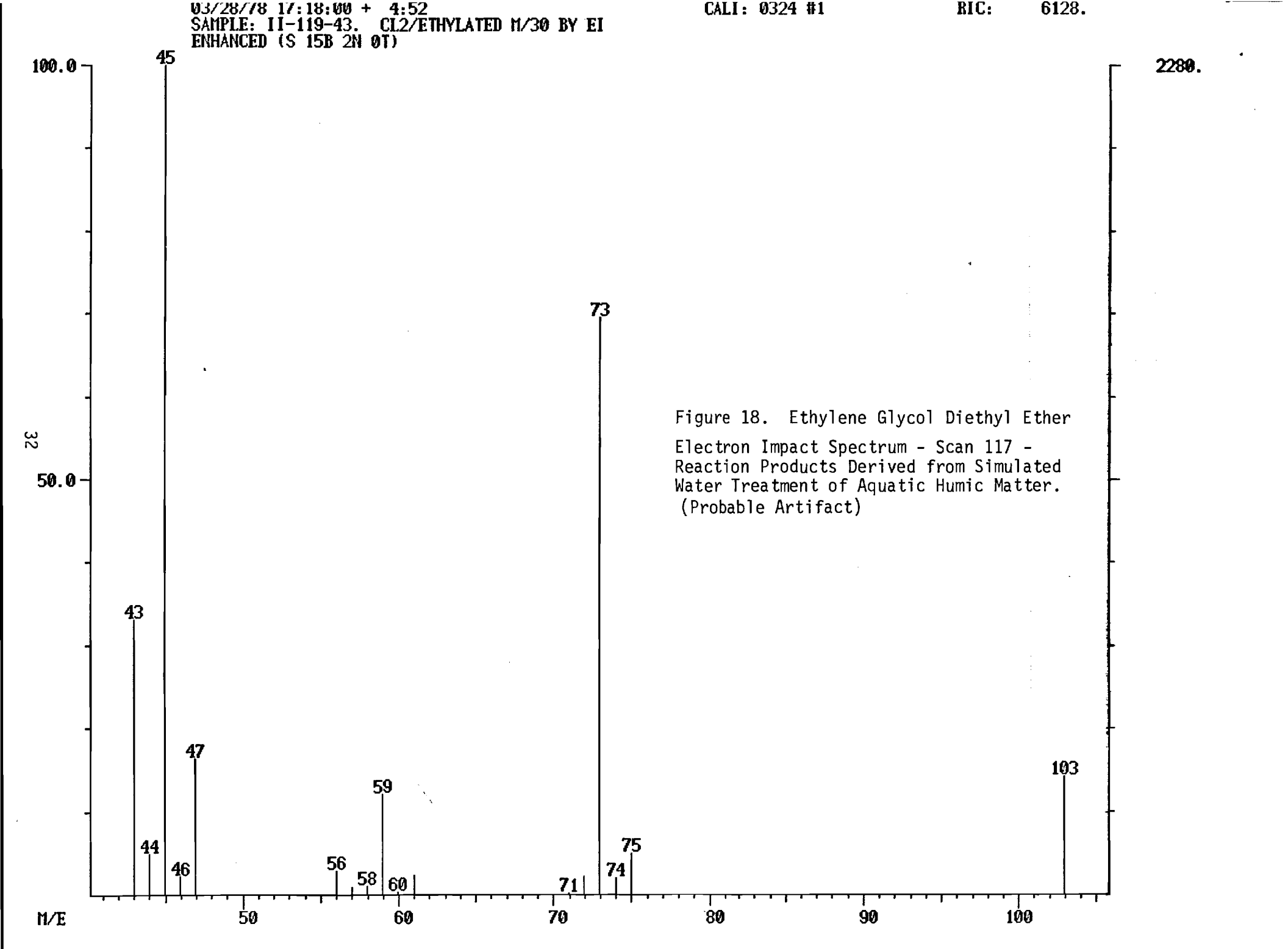


Figure 18. Ethylene Glycol Diethyl Ether Electron Impact Spectrum - Scan 117 - Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter. (Probable Artifact)

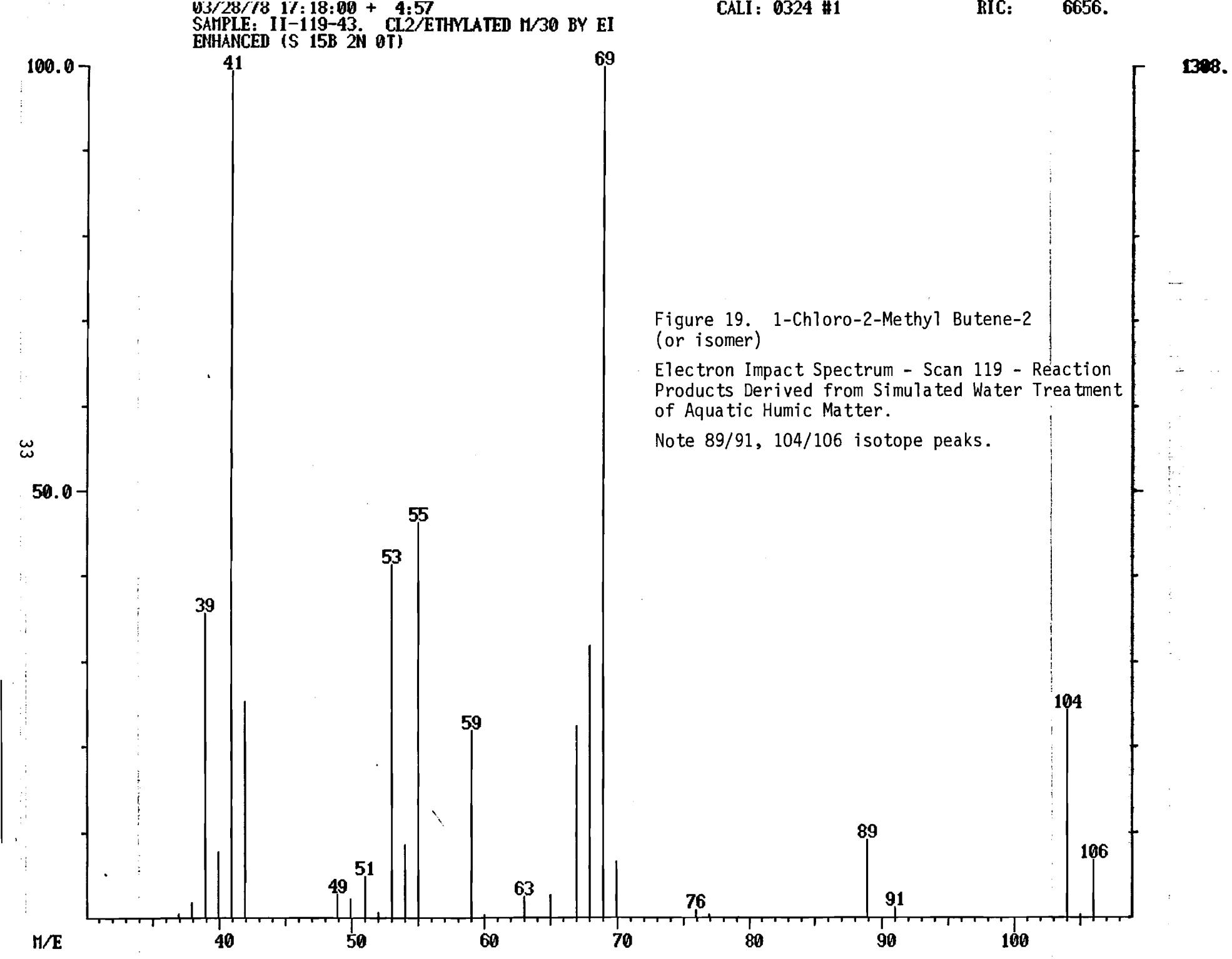


Figure 19. 1-Chloro-2-Methyl Butene-2 (or isomer)

Electron Impact Spectrum - Scan 119 - Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter.

Note 89/91, 104/106 isotope peaks.

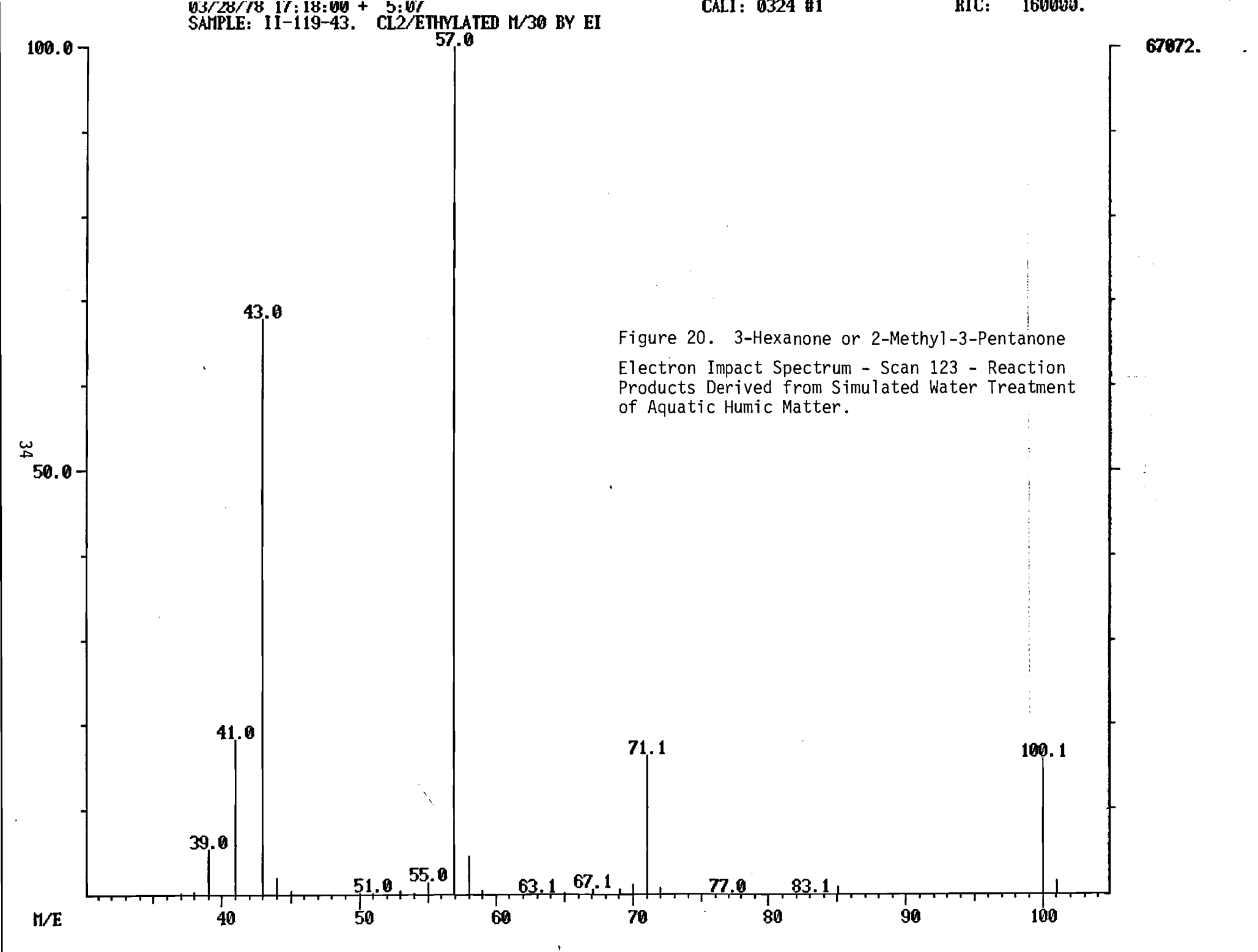


Figure 20. 3-Hexanone or 2-Methyl-3-Pentanone

Electron Impact Spectrum - Scan 123 - Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter.

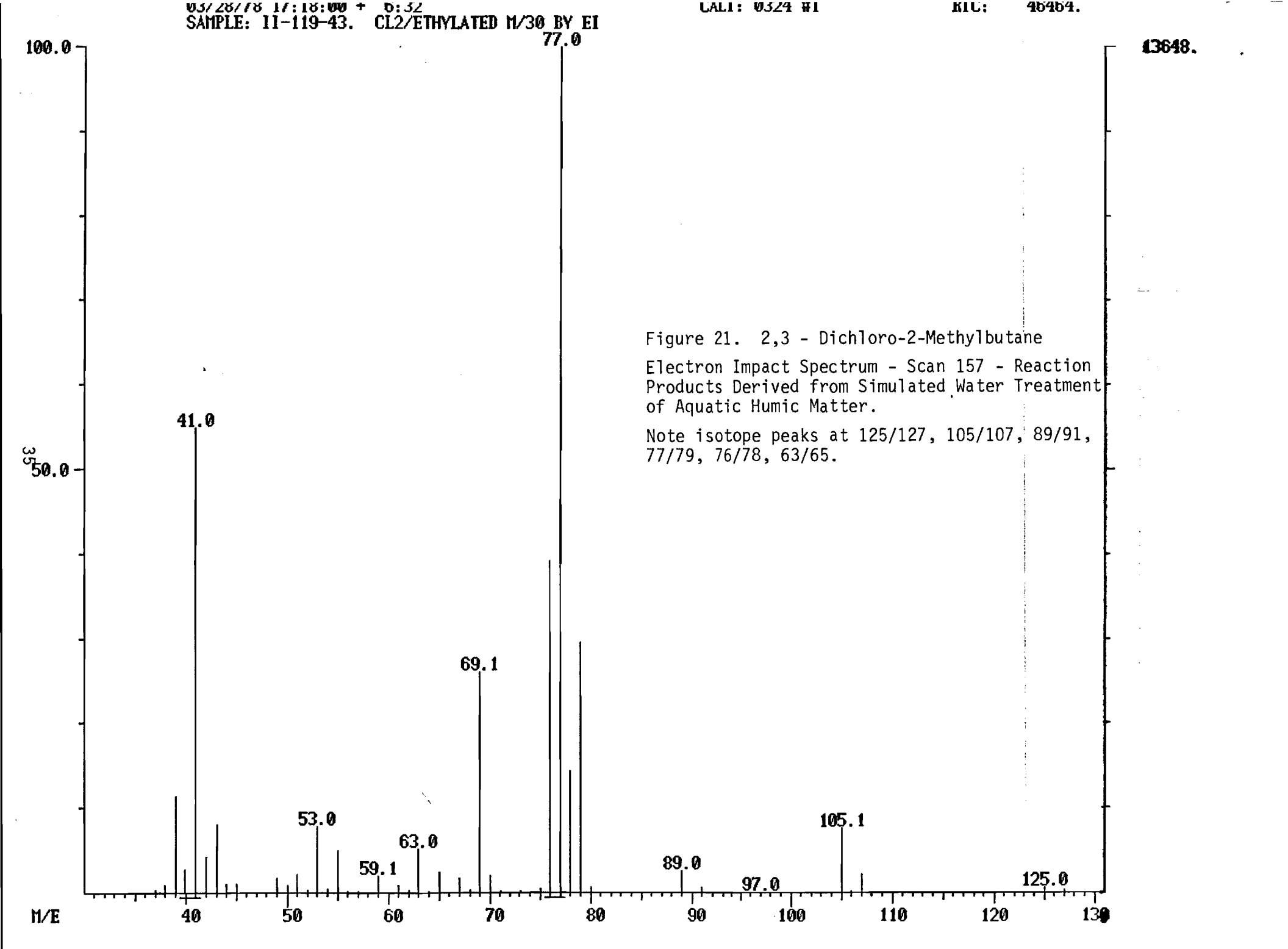


Figure 21. 2,3 - Dichloro-2-Methylbutane

Electron Impact Spectrum - Scan 157 - Reaction Products Derived from Simulated Water Treatment of Aquatic Humic Matter.

Note isotope peaks at 125/127, 105/107, 89/91, 77/79, 76/78, 63/65.

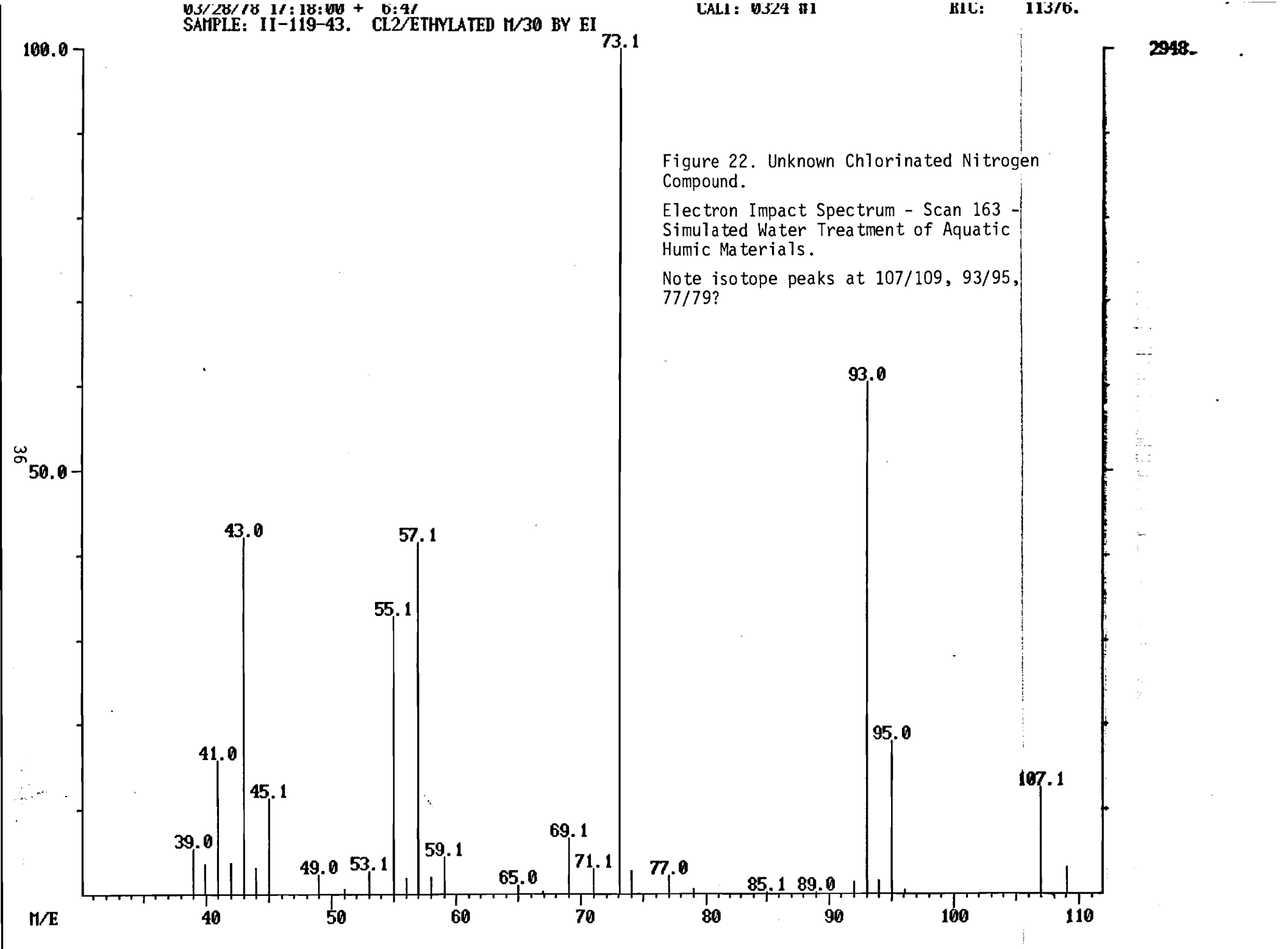


Figure 22. Unknown Chlorinated Nitrogen Compound.

Electron Impact Spectrum - Scan 163 - Simulated Water Treatment of Aquatic Humic Materials.

Note isotope peaks at 107/109, 93/95, 77/79?

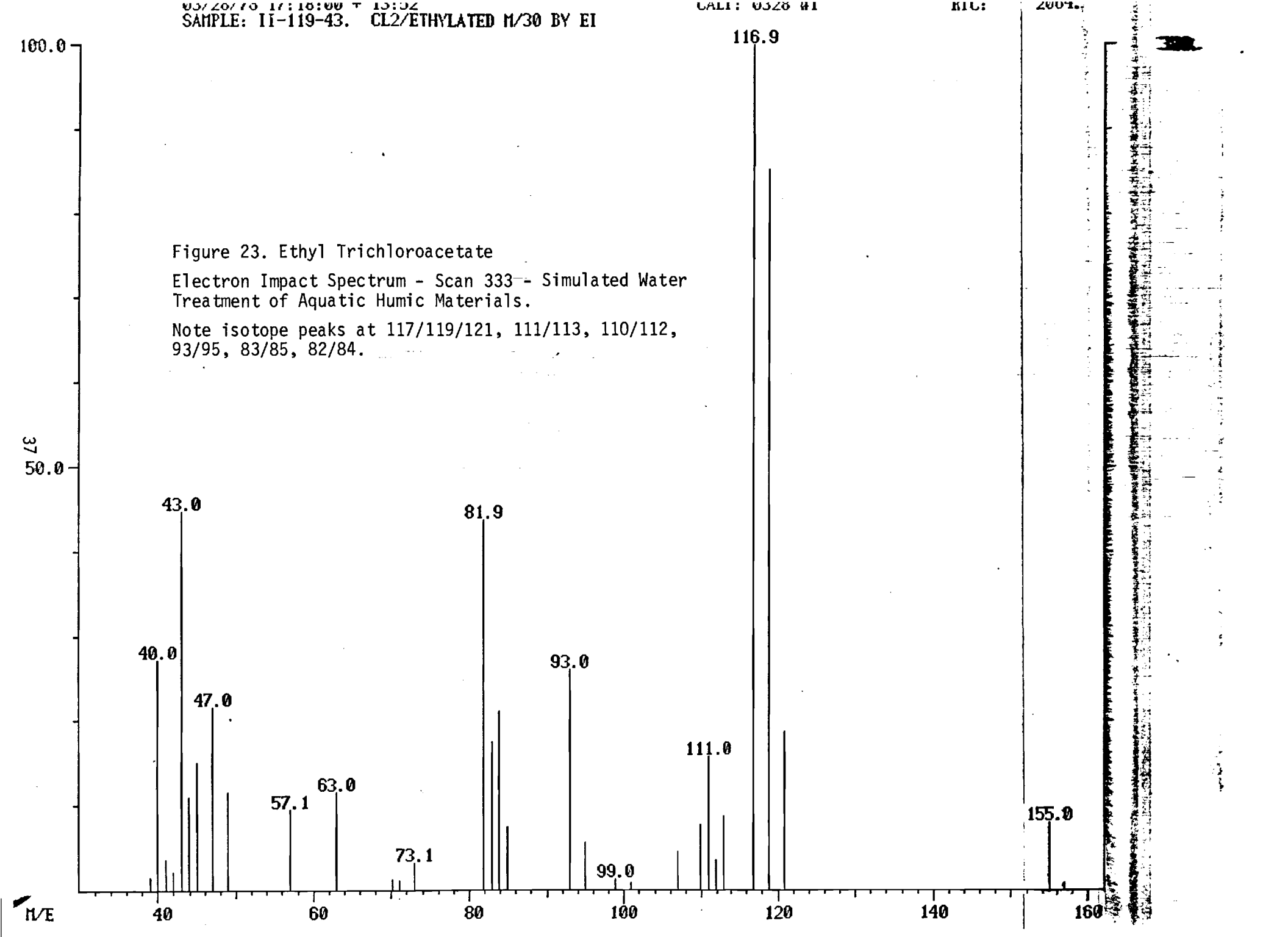


Figure 23. Ethyl Trichloroacetate

Electron Impact Spectrum - Scan 333 - Simulated Water Treatment of Aquatic Humic Materials.

Note isotope peaks at 117/119/121, 111/113, 110/112, 93/95, 83/85, 82/84.

X. WORKUP AND ANALYSES OF VARIOUS RESORCINOL FRACTIONS

It will be recalled from page 32 of the previous report that a solution of resorcinol in highly purified water was treated with chlorine at room temperature for 14 hours after which time, no chlorine residual remained. A control sample containing no resorcinol was treated in the same way. This reaction mixture and control were acidified and passed over beds of XAD-2 resin. These samples were submitted for analysis by x-ray fluorescence. The control sample showed no detectable chlorine. The resorcinol-treated sample was found to contain 1.59 mg of trapped chlorine. Thus the incorporated yield of chlorine was slightly less than 3% of the weight of the original resorcinol or 0.4% of the applied chlorine. The percentage of resorcinol converted to chlorine-containing products is, of course, greater than 3%. For example, if all of the organic chlorine exists in the form of tetrachlororesorcinol, the yield based on resorcinol would be about 5%. It has not yet been possible to confirm the method because the nuclear reactor had been shut down unexpectedly just as our samples were scheduled to go in. Thus they have not yet been analyzed by the neutron activation method. We have been assured that they will be run during the next reporting period.

The reaction products from the mini-pilot work described on page 13 of the February 6, 1978 progress report, which had been stored in the freezer, were subjected to further analysis during this reporting period. The samples examined were:

a) II-81-21 which is a pentane extract from the sand filter effluent

b) II-81-37 which is a pentane extract of the untreated resorcinol solution

c) II-81-28 which is a pentane extract from the settling chamber taken 1.5 hours after the completion of the reaction.

The GC/MS analyses were carried out under the following conditions:

temperature program: hold at 15°C for 4 min, increase at 10°C/min to 150°C and hold 5 min at 150°C.

column: 64 meter glass capillary

stationary phase: SP 2100

split: 12.5 ml/min

sweep: 12.4 ml/min

linear velocity: 30 cm/sec.

Chloroform has been identified in both the sand filter effluent and the settling chamber extracts. The amounts present were large enough to permit detection with no special difficulty. A trace of chloroform was also detected in the control sample, but only if ion mapping techniques were employed. A more quantitative procedure may be employed in the future.

XI. WORKUP AND ANALYSIS OF VARIOUS HESPERETIN FRACTIONS

A similar GC/MS examination of the reaction products resulting from the treatment of hesperetin with chlorine did not produce any information regarding the production of chloroform because both the control and the standard were found to contain chloroform. The source of this extraneous chloroform has now been traced to contaminated pentane. This problem is now being corrected.

XII. GAS CHROMATOGRAPHIC METHODS FOR HALOMETHANES

Attempts to assemble the Lupton detector with a packed chamber and transfer line have not been as successful as had been hoped. Therefore, all current experiments are proceeding without the use of detector packing. The new 60 meter Carbowax 20M and SP2100 capillary columns have proved to be extremely effective for the separation of a standard mixture of chloroform, bromochloromethane, carbon tetrachloride, bromodichloromethane, dibromochloromethane, bromoform and 1,4 dichlorobutane. Figure 24 shows

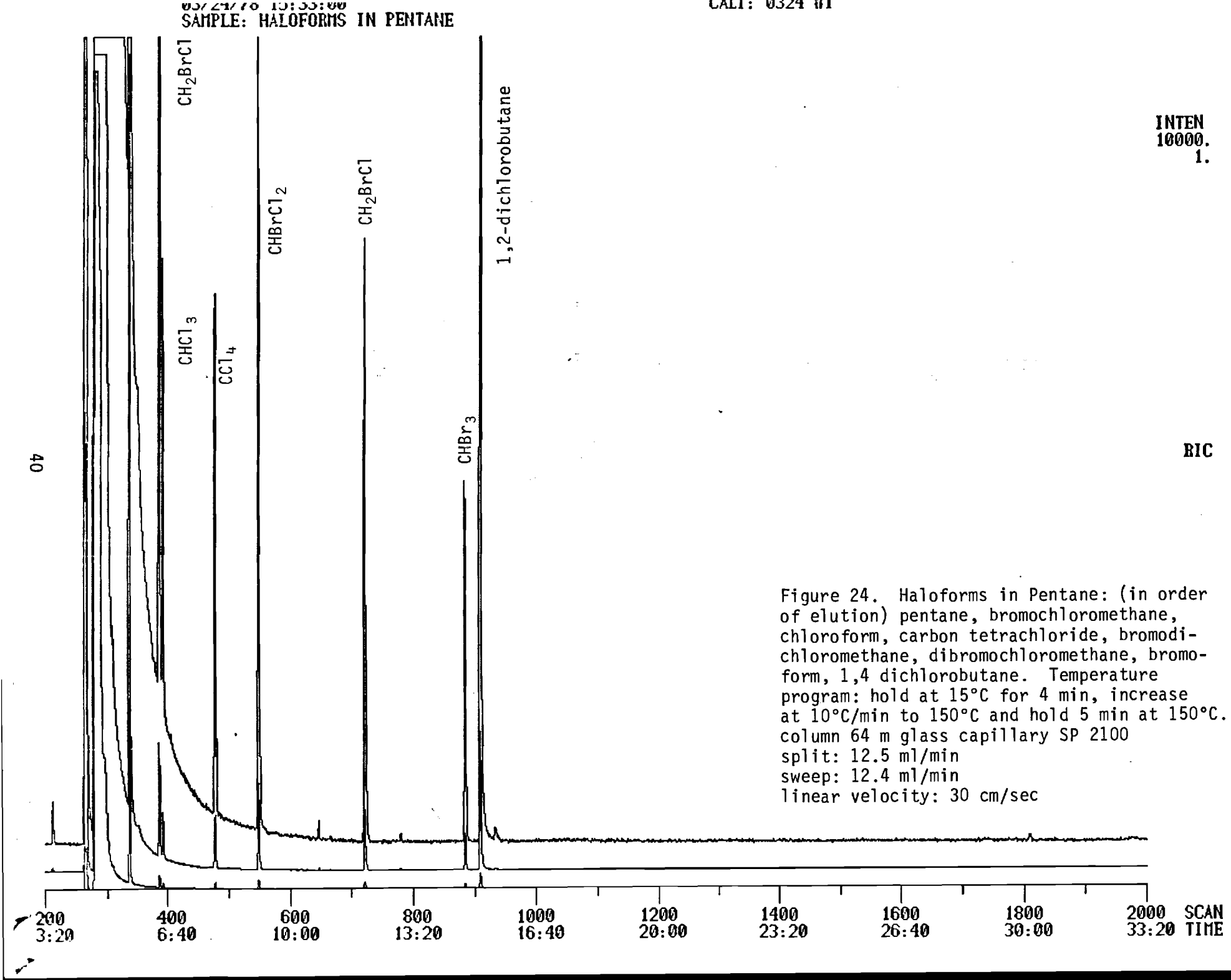


Figure 24. Haloforms in Pentane: (in order of elution) pentane, bromochloromethane, chloroform, carbon tetrachloride, bromodichloromethane, dibromochloromethane, bromoform, 1,4 dichlorobutane. Temperature program: hold at 15°C for 4 min, increase at 10°C/min to 150°C and hold 5 min at 150°C.
column 64 m glass capillary SP 2100
split: 12.5 ml/min
sweep: 12.4 ml/min
linear velocity: 30 cm/sec

the degree of separation achieved with the SP2100 column. The Carbowax column was slightly more effective. This work was done using the mass spectrometer as the detector. A single attempt to use the Lupton detector directly coupled to a capillary column was less than satisfactory. It would seem that miniaturization would be required if this objective is to be achieved.

A 6 ft × 2.5 mm column packed with Ultrabond 20M 100/120 mesh was found to be capable of separating a mixture of carbon tetrachloride, chloroform, bromodichloromethane, dibromochloromethane and bromoform under the following conditions:

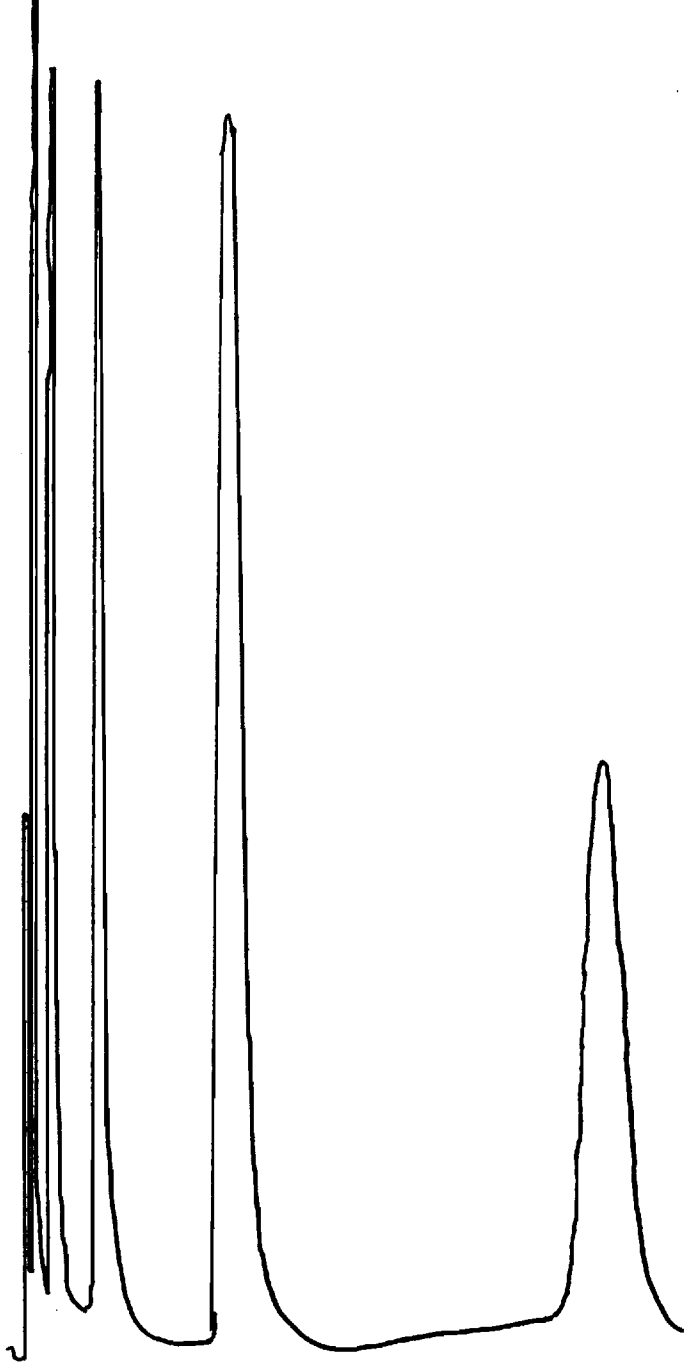
column temperature 25°C
cathode voltage 0.915 V
buck out 2.0 volts
nitrogen 45 psig, ml/min
"standing current" 2.0×10^{-9} amps
sensitivity 1×10^{-10} AFS

These results are shown in Figure 25.

A 12 ft column was packed in a successful effort to further improve the separation of the early peaks. Standard solutions of the halomethanes will be used to establish conditions of optimum detector performance during the next reporting period.

XIII. LIQUID CHROMATOGRAPHIC SEPARATION OF HESPERETIN AND ITS CHLORINATION/OXIDATION PRODUCTS

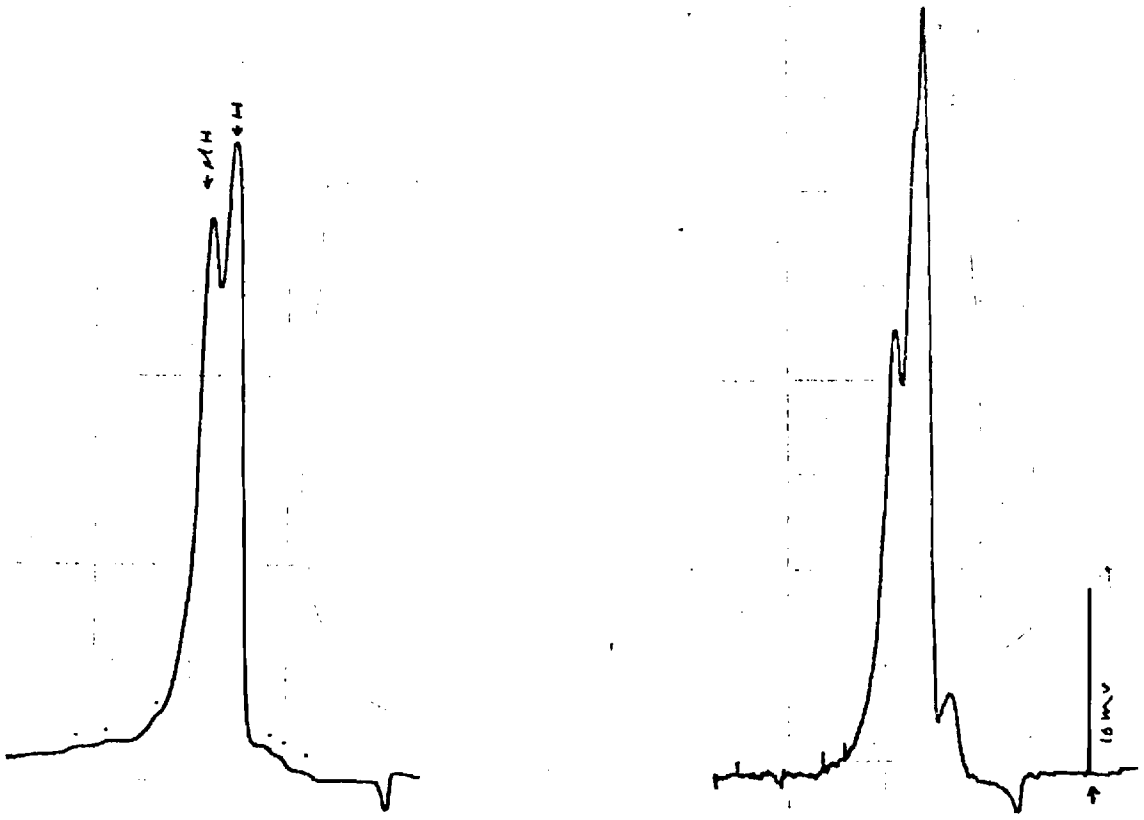
It will be recalled that a gradient elution technique had been successfully applied to the analysis of hesperetin from two different sources. Using a 0.03M potassium dihydrogen phosphate buffer system, it was possible to detect tiny traces of iso sakuanetin as an impurity in a sample obtained from the Coca Cola Company but not in a sample provided by the USDA.



column: 6 ft × 2.5 mm glass
packing: 100/120 mesh ultrabond 20 M
carrier: N_2 at 45 psig

Figure 25. Haloforms in pentane (in order of elution) pentane, carbon tetrachloride, chloroform, bromodichloromethane, dibromochloro methane, bromoform.

A sample of methylated hesperetin was examined by LC methods using a 10% tp 100% methanol-in-water gradient. The solutions were buffered with 0.03 M potassium dihydrogen phosphate. The reaction product mixture was adjusted to a concentration of 3 mg/l in ether. The injection size was 1.0 ml, the detector was set at 289 mm. Two overlapping peaks were observed, the first of which had a retention volume of 2.5 ml. The column employed for the separation was a (5mm×25cm) Vydac 201 TP reverse phase. It was therefore concluded on the basis of the evidence shown below (left) that the methylation reaction had not gone to completion.



Less Complete Methylation

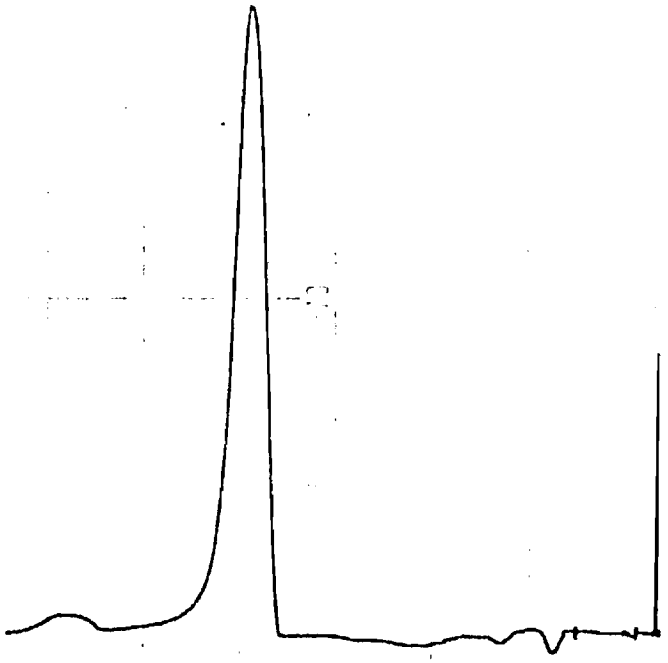
More Complete Methylation

Continued reaction of this material with diazomethane resulted in the shrinkage of the hesperetin peak and the development of two shoulders on

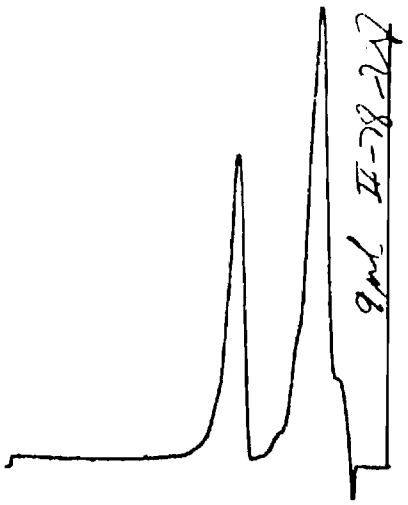
the "methylated" hesperetin peak. It seems likely that only the most strongly retained shoulder represents fully methylated hesperetin (retention volume 3.1 ml) while the other two represent the mono and dimethyl hesperetins, respectively (above right). The same general conditions were also employed to determine that some of the hesperetin in the above reaction mixtures was being retained on the sodium sulfate used for drying the product mixtures. A more thorough washing procedure has been instituted to correct this problem.

Portions of the product mixture resulting from the chlorination of hesperetin in the mini-pilot facility under the conditions described on page 33 of the previous monthly report were subjected to 2c analysis in the methanol-water-dihydrogen phosphate gradient system described earlier in this section. The control containing hesperetin, buffer and no chlorine was extracted with pentane at a pH of 10.2. The product mixture was treated in a similar manner. Neither of the extracts showed any absorbance at 289nm during the course of the analysis.

The pH was adjusted to 1.3 and further extractions were carried out with ethyl ether. In this case, the control showed the hesperetin peak with the expected retention volume of 2.5 ml. The reaction mixture showed two completely separated peaks at retention volumes of 1.9 and 3.8 ml, respectively. The first peak had several shoulders. These results are presented below.



Control (Faster Chart Speed)



Product Mixture (Slower Chart Speed)

The ether extracts derived from the settling chamber showed a similar elution pattern. It is interesting to note that the molecular structure of the hesperetin is preserved since the UV spectra recorded for each of the two components are not very different from that of hesperetin itself. Extracts from a second separate reaction were essentially the same.

XIV. REACTION OF CHLOROFORM WITH CHLORINE

A preliminary reaction has been carried out using a chloroform solution having a concentration of 1.5 mg/l and 200 ml of a solution containing 200 ml of chlorine at a concentration of 2.0 mg/ml. A control was set up in the same manner except that 200 ml of high purity water was added in place of the chlorine solution. Aliquots (100 ml) were withdrawn periodically and extracted with pentane (2×25ml). The pentane extracts were subsequently dried

over sodium sulfate and concentrated on a Kuderna Danish apparatus. An analysis of this material is now in progress. Unless these extractions predate the contamination of the pentane and/or the concentration changes are dramatic, it is likely that this work will have to be repeated.

XV. SUMMARY AND PLANS

The total acidity of two samples of aquatic humics was estimated thus concluding work which had begun during the last reporting period. An improved method was developed for the estimation of carboxyl groups in aquatic humics by the acetate method. Milder conditions were employed for the oxidation of aquatic humics with permanganate. In spite of the collection of a steam distillate, more than 2.5 times as much organic material was recovered. GC/MS analyses of these oxidation product mixtures has been facilitated by the use of our new high-quality 60 meter capillary columns. Some of the details of this work have been presented in tabular form. Individual spectra have been provided for selected products.

Jar tests were conducted with solutions of aquatic humic matter in order to establish optimum conditions for floc formation. Tests with the chlorine generator confirmed that no carryover of hydrochloric acid had occurred. The reaction of aquatic humics with chlorine in the mini-pilot facility has resulted in the production of a large number of products--many of which contain chlorine. This aspect of the work is believed to be highly significant and will be pursued with vigor.

A workup of the reaction products arising from the chlorination of resorcinol have been examined by GC/MS. Chloroform has been identified in the volatile, neutral fraction. A problem dealing with the contamination of high-purity solvents has been detected and is being corrected.

High quality separations have been achieved for the halomethanes using

both packed and capillary columns. Although the Lupton detector functions at low flows, miniaturization of the detector would seem likely to improve performance under these conditions. Gradient elution HPLC has been successfully applied to the separation of reaction products derived from the chlorination/oxidation of hesperetin. Several products have been noted which bear a structural relationship to the starting material as evidenced by the similarity of their UV-VIS spectra. Such materials could be of interest if they are also found to contain chlorine. Ion pair chromatography will be applied to these products as soon as the reagents arrive.

The reaction of chloroform with chlorine was inconclusive and will be repeated in the near future.

Experiments with carbon beds and with the reaction of aquatic humics in the presence of flocculating agents will be conducted using the mini-pilot facility during the next reporting period. The backlog of reaction products from earlier experiments will be further reduced. The reaction of chlorine with aquatic humics will receive special attention in the coming weeks. Class separation experiments will begin with specially designated samples of Satilla River water to be withdrawn during the next reporting period.

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Monthly Progress Report for May 1978

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUNO IN DRINKING WATER

Monthly Progress Report

June 9, 1978

by

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68-01-4480

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*Principal Investigator to whom inquiries should be directed.

ENGINEERING EXPERIMENT STATION 225 North Avenue, Northwest · Atlanta, Georgia 30332

June 9, 1978

Dr. Charles Trichilo
Environmental Protection Agency
401 M Street, S.W.
Office of Water Supply (WH-550)
Criteria and Standards Division
WSME, Room 1030
Washington, D.C. 20460

Subject: Monthly Progress Report
"Identification of Major and Minor Classes of Natural Organic Substances Found in Drinking Water"
Contract No. 68-01-4480
Georgia Tech No. A-1983-000

Dear Chuck:

Enclosed is a copy of this month's progress report. We have made substantial progress in the characterization of aquatic humics via a milder permanganate oxidation procedure, have added several minipilot runs of aquatic humics and have begun a series of class separations with raw river water. It is always difficult to document all of the progress in a single report, so we have devised a plan for organizing and presenting new data on continuing experiments in order that the reviewers will not be confused by seeing only that part of the data which was collected during a single reporting period. We solicit your comments and suggestions regarding this matter.

Helmut and I feel it is our duty to inform you that Georgia Tech's overhead rate has been increased from 68% to 76%. It has been explained to me that while this change in no way invalidates our contract, the new rate will be applied as of July 1. Therefore, if you desire to maintain the same level of effort, you can ask your financial people (who have already been notified directly by Georgia Tech) that you desire to increase our remining personnel budget by 8%. Alternatively, we could submit a proposal for an add-on which would include enough money to cover the shortfall and which would specify work in some new areas not covered by the current proposal such as studying the nature of the compounds associated with aquatic humics (as opposed to those actually involved in the structure), algal studies concentrating on extracellular materials and minipilot studies with BrCl, I_2 and other disinfectants.

I will be on vacation for the next two weeks. Therefore, if you have any questions, please direct them to Dr. Ralls and/or Dr. Reuter.

[illegible] me in case of an especially pressing need.

Sincerely,

S. C. Havlicek, Ph.D.
Principal Investigator
A-1983-000

DISCLAIMER

This report has not been reviewed by the Criteria and Standards Division, Office of Water Supply, U. S. Environmental Protection Agency, and is intended for internal circulation only. The contents do not necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

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EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastwater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources. Considerable progress has been made in characterizing this material. Milder oxidation procedures developed over the past two reporting periods have resulted in fivefold increases in the yields of oxidative degradation products. Workup and the acquisition of GC/MS data have been completed. The spectra of all significant components in the mixture were subjected to interpretation. These results continue to support a more open chain structure than has been traditionally assigned to aquatic humic substances.

Significant progress has been made during this reporting period regarding the identification of the products resulting from the chlorination of aquatic humic substances in our minipilot water treatment facility. More than 15 components have been characterized using the complementry techniques of chemical ionization (CI) and electron impact (EI) mass spectrometry in conjunction with capillary column gas chromatography. Five runs have been made to date. In three of the five runs, significant numbers of chlorination products have been found. Of those identified in the treated water, more than half contain chlorine. All of the compounds identified in the control samples were also found to be present in at least one of the reaction mixtures. The isoprene skeletal framework continues to dominate the

structural assignments. New work in this area will concentrate on correlating data from run to run as well as within a run, certifying structural assignments by comparison with authentic samples and the use of milder chemical ionization reagent gases.

The application of the new on-column Lupton electron capture detector to the analysis of halogenated organic reaction product mixtures represents a fundamental advance in our analytical capabilities and will be actively pursued during the next reporting period.

Raw water from the Satilla River has been separated into classes using sequential adsorption on XAD-8, XAD-2, AG MP-50 and AG MP-1 resins. A material balance has been recorded using TOC as a measure of the amount of material in each class. The results of these determinations indicate that the hydrophobic bases account for about 5-10% of the TOC, hydrophobic acids 45%, hydrophilic bases less than 5%, hydrophilic acids 25%, and hydrophilic neutrals 10-15% of the TOC.

A subsample of this raw river water was treated with chlorine and worked up in the usual manner so that the results of the GC/MS analysis can be compared with those obtained for the aquatic humic substances. Jar tests have shown that ferric chloride flocculation can reduce the TOC levels by a factor of 3. A rerun of the river water minipilot experiments using the conditions suggested by the jar tests is planned.

Paired-ion liquid chlromatography has been shown to be effective in resolving both standard and product mixtures. This techniques, perhaps in conjunction with our new high-resolution, reversed-phase column should enable us to devote more attention to the characterization of non-volatile and hydrophilic fractions.

An additional sample of raw river water was taken during the reporting period and is in the final stages of processing. A second bromination reaction of aquatic humic substances was undertaken and worked up for future GC/MS analysis.

An improved test procedure based on the oxidation of Mn^{+2} for active oxygen species is being optimized so that the outputs of various types of ozone generators may be more thoroughly characterized. It is evident that Mn^{+2} concentration, pH, temperature, reaction time, presence of O_2 and perhaps even the time elapsed between the generation of the active

oxygen and its entry into the test solution may all be important factors.

Further work on the estimation of the carboxyl content of aquatic humic matter has made it evident that the ratios of carboxylic to phenolic acidity currently published in the literature are twice as high as they should be. This discovery suggests that our ideas about the close chemical relationship between river water humics and soil fulvic acids may have to be revised.

I. PERSONNEL

No changes in personnel have taken place during the reporting period. A promotion for Dr. El-Barbary is being processed which will transfer him out of our laboratory. Candidates have already been interviewed to serve as his replacement. In the meanwhile, the rest of the staff will pick up his share of the workload so that we can maintain our momentum on the LC work.

II. EQUIPMENT

The GC/MS and other major instrumentation continue to function smoothly. Such difficulties as have been encountered have been resolved by our own staff at an early stage. Thus our only downtimes have been those required for normal maintenance such as cleaning and realigning the source. The last source realignment appears to have brought about a significant increase in sensitivity which, if it continues, should help us identify minor constituents with greater efficiency.

The Continental Water conditioning unit has been replaced with a new unit containing a second activated carbon filter. This replacement was undertaken on the basis of experimental evidence indicating breakthrough of chloroform. Since such a breakthrough had been expected, the writers had been distilling the high-purity water and throwing away the first 10-20% in order to minimize THM carryover. Occasionally, the volumes of water required have made the performance of this extra step inconvenient, so it was considered to be worthwhile to institute the changes.

III. CLASS SEPARATION OF SATILLA RIVER WATER

A 4.4-liter subsample of Satilla River water collected during this reporting period was filtered through Whatman No. 1 paper to remove the small amounts of suspended solids which had built up in the sample during transit. Portions of this filtered subsample were retained for TOC and purge-and-trap analysis. The bulk of the subsample (3.9 liters) was placed in a glass bottle for closed circuit stripping of volatile constituents into activated carbon (0.102 g, DARCO S-51, 4-12 mesh). An equilibrium technique adapted from K. Grob[1] was employed. A portion of the water remaining after this treatment was retained for TOC and purge-and-trap measurements. In this way, the TOC will provide a check on the material balance.

The remaining water sample (3.3 liters) was treated with 10 drops of concentrated potassium hydroxide in order to bring the pH to 9.0. This water (3.3 liters) was passed through four beds (11 x 50 mm) of precleaned XAD-8 macroreticular resin. Each bed contained about 2 g of resin (four beds were used to speed the completion of the process without introducing an unduly rapid flow rate). In this way, the flow rate was maintained at 110 ml/hour/bed.

A sample of the XAD-8 resin column eluate was reserved for TOC measurement. The remaining eluate (3.1 l) was passed through four beds of XAD-2 resin in a similar way as that described for the XAD-8 treatment. A sample of the eluate (pH = 7.5) from the XAD-2 resin was retained for TOC measurement. At this point in the separation-concentration sequence,

---

[1] K. Grob, J. Chromatography, 84, 255 (1973).

(see Figure 1) the hydrophobic bases should have been retained on the resin beds. The XAD-8 and XAD-2 beds were then backwashed with 500 ml 0.1 N hydrochloric acid per bed, thus providing 2 l of separated hydrophobic acids from each type of resin.

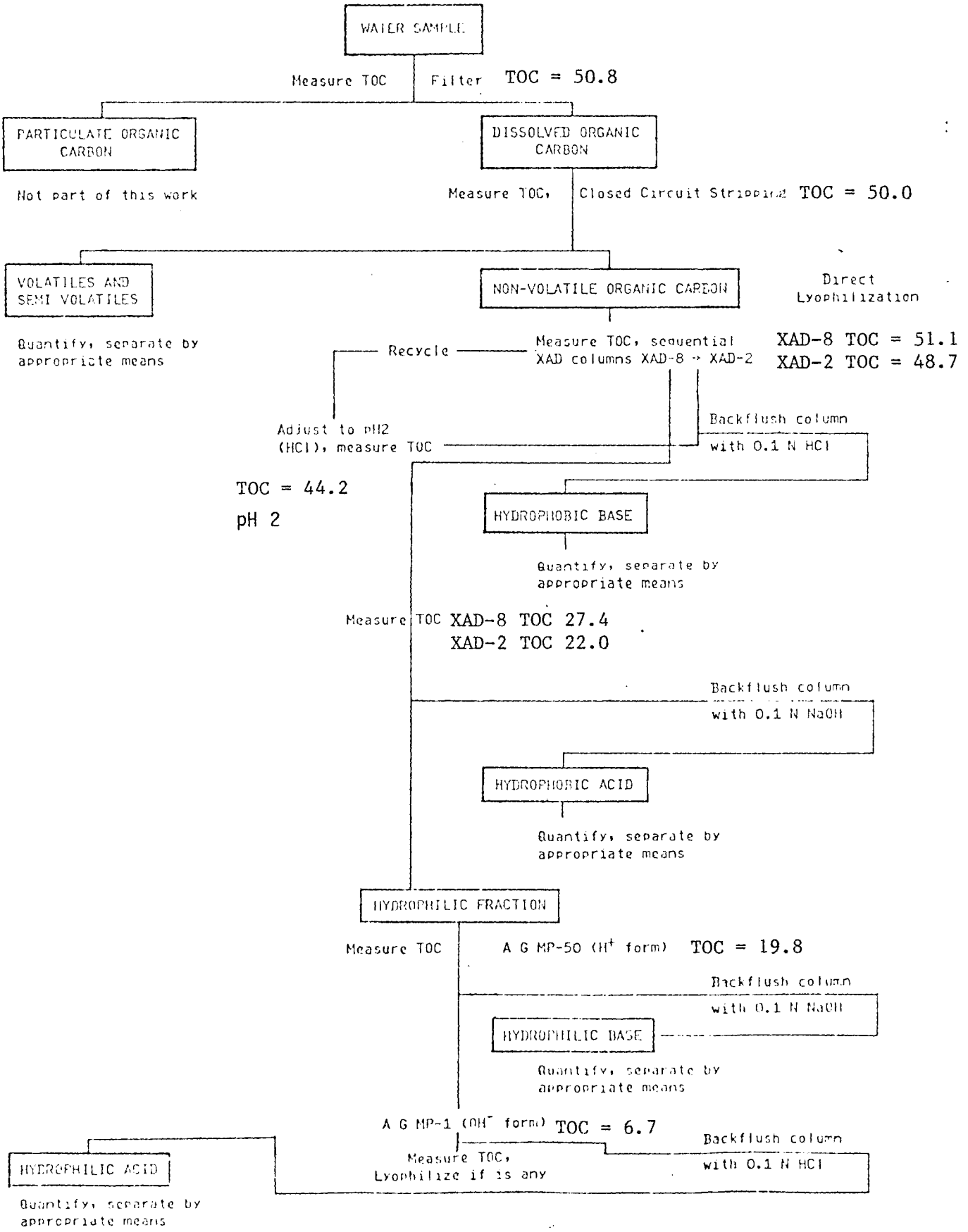
The pH of the XAD-2 resin eluant was adjusted to 2.0 with purified 6N hydrochloric acid. A brown precipitate slowly developed in this solution (2.8 l) as it was passed through the four recycled beds of XAD-8 in the manner described above. As a result of the precipitate formation, the flow rate dropped to only a few ml/hr after about 2 l of water had passed through the resin. Once again a portion of the eluate was retained for TOC determination.

The remaining eluate (2.3 l) was subsequently passed through the recycled XAD-2 beds which removed very little additional colored material. A portion of this eluate (pH 2.1) was also retained for TOC evaluation.

Two beds of pre-purified AG MP-50 ion exchange resin (H+ form) equivalent to 4 g of dry resin were prepared in water, following activation with 10 ml of 3N purified hydrochloric acid and washing with 100 ml distilled water per bed, the columns were treated with the remaining XAD eluates at a flow rate of 125 ml per hour per bed. A portion of this eluate (1.9 l, pH 1.2) was retained for TOC analysis. The remaining eluate was brought to pH 7.0 with 1N NaOH (65 ml).

Two columns of pre-purified AG MP-1 resin containing about 2 g each of material on a dry weight basis were converted to the OH-form with 10 ml of 1N sodium hydroxide solution and washed with 200 ml of distilled water. The remaining 1.6 l of eluate was passed through the resin beds at a flow rate of 150 ml/hour/bed. The color was completely removed by

Figure 1. Separation-Concentration Scheme



this treatment. The upper part of the resin became dark brown. A portion of this eluate (1.5 l, pH 7.0) was retained for the measurements. This eluate contains the hydrophilic neutrals.

Back-flushing of the AG MP-50 and AG MP-1 beds with a total of 200 ml of 0.1N sodium hydroxide and 0.1N sulfuric acid provided the hydrophilic base and hydrophilic acid fractions.

TOC determinations were carried out by Dr. Fisher Craft using his Beckman 915 TOC Analyzer. The precision of the method for the 10-50 mg/l range has been established as ± 0.5 mg/l. The appropriate TOC values are noted on Figure 1 next to each of the steps. In this way it can be seen that hydrophobic bases account for about 5-10% of the TOC, hydrophobic acids 45%, hydrophilic bases less than 5%, hydrophilic acids 25% and hydrophilic neutrals 10-15%.

IV. TREATMENT OF RAW WATER FROM THE SATILLA RIVER IN THE MINI-PILOT FACILITY

In accordance with our evolving attention to matters of quality control, the components of the mini-pilot facility were rinsed with several portions of deionized-distilled water and dried for a minimum of 2 hours in an oven at 255^{o}C. The reservoir bottle which was too large to fit into the oven was rinsed with deionized-distilled water, drained completely, and dried with a nitrogen gas flow.

Ten liters of unfiltered Satilla River water was placed in the reservoir bottle and 320 mg of "roasted" calcium hydroxide added. After the calcium hydroxide had dissolved, the pH of the water was found to be 7.1. The river water, which was brown in color, was added to the mixing chamber at a rate of 1 liter/hour. A chlorine-aluminum sulfate solution was added to the missing chamber at a rate of 50 ml/hour, the concentration of aluminum sulfate was 0.2 g/100 ml and the chlorine concentration (generated in three separate portions) was 180 mg, 205 mg, and 290 mg/ 100 ml. The total chlorine added was 880 mg. The stirring rate in the mixing chamber was 160 RPM. A brown floc settled out in the baffled agitation section of the mini-plant after thirty minutes of operation. The color of the water after flocculation and chlorination was considerably lighter than that of the raw water but was still noticeably yellow. The flow from the settling chamber into the sand filter began after 5.6 hours of operation. A water sample taken at this point was yellow, had a pH of 3.4 , and did not show a chlorine residual.

At the end of an eight-hour period of operation, the yellow water in the settling chamber still had no residual chlorine. The pH was found to be 3.7.

It is unlikely that the raw water of this quality would be used directly in commercial water treatment practice due to the high chlorine demand and high total organic carbon of 51 mg/l. Nevertheless, it is a good "worst case" test and should provide much useful information—especially when repeated with higher chlorine dosages. A subsequent jar-test with ferric chloride produced a heavy, brown colored precipitate which judging by the appearance of the solution should cause a substantial reduction in TOC and chlorine demand. Pre-treatment of raw Satilla River water with ferric chloride prior to chlorination would represent a condition closer to commercial practice in water treatment. A mini-plant run using ferric chloride-treated Satilla River water has been scheduled for the next reporting period.

V. SATILLA RIVER JAR-TESTS

1. All glassware was rinsed in purified water prior to use. Raw Satilla River water (1 l, pH 4.1) was transferred to a standard jar. Calcium hydroxide (17 mg) was added with stirring to bring the pH up to 7.0. Aluminum sulfate (25 mg) was next added with stirring (180 RPM). Since no visible change in the sample was noted, another 25 mg $Al_2(SO_4)_3$ was added under the same conditions. Although some cloudiness was observed, no floc formation occurred. Still another 25 mg $Al_2(SO_4)_3$ was added as before. In this case, the sample became extremely turbid but floc did not settle out at a visible rate. The pH had dropped to 4.0 at this point. Yet another 25 mg $Al_2(SO_4)_3$ was added, again under the same conditions, producing further increases in turbidity. The pH was found to be 3.8. In this case some settling was observed after 20-30 minutes.

   The addition of 100 mg kaolin shortened the settling time somewhat (20 minutes). Following this treatment the pH was found to be 4.3. The addition of 15 mg calcium hydroxide was sufficient to raise the pH to 7.0.

2. A second sample of river water (1 l, pH 4.1) was transferred to a precleaned sample jar and treated with four successive 25 mg quantities of $Al_2(SO_4)_3$. Stirring was applied at 180 RPM. A slight degree of flocculation was observed after the last addition.

3. A third sample of river water (1 l) was transferred to a precleaned jar and treated with 25 mg of $FeCl_3$, with stirring. Although the solution darkened and developed some cloudiness, no floc was observed. The application of an additional 25 mg of $FeCl_3$ produced

a further darkening and cloudiness with floc formation. This floc required approximately 15 minutes for settling and resulted in a dramatic clarification of the raw water. The pH had dropped to 3.1 during this treatment. The TOC value for the flocculated sample was 17 mg/l as compared to 51 mg/l for the raw water.

VI. LIQUID CHROMATOGRAPHY

The applicability of paired ion chromatography (PIC) for the liquid chromatographic separation of dichloroacetic acid and trichloroacetic acid potentially formed from the chlorination of model compounds and aquatic humics was studied in some detail. The organic counter ion used in this study was tetrabutylammonium phosphate; when this reagent is dissolved in a phosphate buffer at pH 7.5, both strong and weak acids which are in an ionized form at this pH intereact with the tetrabutyl ammonium ion to an extent which is dependent, at least in part, on their acid strength. The associated form being more organic and less hydrophilic in character will be retarded to a greater degree by a hydrophobic LC stationary phase. Hence the generally superior separating powers of reversed phase LC can be used in place of anion exchange LC methods. Thus we have been eager to apply this method to our work because organic acids are expected to be major products in both our oxidative degradation studies and in our studies with Cl_2, ClO_2 and O_3.

Early in this study it was found that the solvent-buffer mixtures containing the paired-ion reagent must be filtered through 0.45 μm membranes to remove extremely fine colloidal particles which otherwise may ruin the column and/or attenuate the UV-VIS light passing through solutions in the detector cell. Isocratic systems were used to develop conditions for the separation of dichloro- and trichloroacetic acids. Unless otherwise noted, the operational conditions for the liquid chromatographic unit were:

Wavelength	254 nm
Flow Rate	0.4 ml/min.
Pressure	1000 psi
Column	VYDAC TP 201 Reverse Phase
Column Dimensions:	25 cm x 5 mm

A solvent mixture of 95:5 (V/V) methanol-water containing the tetrabutylammonium phosphate gave a single peak with a retention volume of 0.7 ml for both acids and a mixture of the two. It might be noted at this point that the presence of the butylammonium group apparently enhances the UV absorbance at 254 nm which is otherwise not a particularly good wavelength for carboxylic acids. No resolution of the two acids was obtained with methanol-water mixtures (all containing tetrabutylammonium phosphate) of 90:10, 80:20, 70:30. Partial separation was obtained with methanol-water mixtures of 60:40, 50:50, 40:60 and 35:65.

Resolution of the mixture of acids was obtained with methanol-water in the ratio of 20:80 (V/V) containing 0.05 M tetrabutyl ammonium phosphate. For these separations, the chart speed was 8"/min and the flow rate 1.8 ml/min. The trichloroacetic acid standard showed two well resolved peaks with retention volumes of 2.3 and 7.7 ml. The dichloroacetic acid standard showed three peaks with retention volumes of 2.3, 3.6 and 6.3 ml. The mixture of the two acids gave four peaks with retention times of 2.3, 3.6, 6.2 and 7.7 ml.

The standards used were reagent grade and may be mixtures of mono-, di- and tri- chloroacetic acids. The results suggest that the peaks at 2.3, 3.6 and 6.2 ml retention volumes are mono-, di- and tri- chloroacetic acids, respectively. The peak with a retention volume of 7.7 ml appears to be due to a reaction product of trichloroacetic acid and a component of the solvent system. A concentrated solution was checked visually after standing for a few days and was found to have two layers, an aqueous layer and a heavier organic layer. The fractions were separated for further analysis by GC/MS.

A dichloroacetic acid standard in acetonitrile showed two peaks with a shoulder on the second peak. The retention volumes were 1.2 and 1.7 ml, respectively. The trichloroacetic acid in acetonitrile showed a small peak at 1.2 ml and two large peaks at 1.7 and 4.8 ml retention volume. The mixture of the two acids in acetonitrile showed three peaks with retention volumes of 1.2, 1.7 and 5.3 ml. The last peak corresponds to the trichloroacetic acid peak. The retention volume is believed to have been shifted slightly by the influence of the other components of the mixture. Fractions from the work-up of a chlorination of aquatic humic material were analyzed for organic acids using the liquid chromatographic conditions described above. The chlorination of the M/30 isolate of aquatic humic material was carried out as described in the April 1978 monthly report, page 25. Experimentally the reaction conditions differed slightly, but these changes are not considered important to this discussion. The ethyl ether extracts of the "mini-plant" fractions were analyzed by liquid chromatography prior to treatment with diazoethane.

The operating conditions were as described earlier. Methanol-water (20:80) containing 0.05 M tetrabutylammonium phosphate was employed as the developing solvent. A concentrated extract of the control sample showed only a slight elevation from baseline at a retention volume of 2.1 ml. This is very weak, however, and would obscure only very small quantities of dichloroacetic acid.

Substantially larger peaks were obtained from concentrated extracts of chlorinated fractions. The sand filter eluate sample showed two peaks with retention volumes of 1.2 and 2.1 ml corresponding to mono- and dichloroacetic acid. The settling chamber concentrate showed a single

broad peak with a retention volume of 2.1 ml which corresponds to dichloroacetic acid.

These liquid chromatographic results strongly suggest that chlorinated acetic acids are products of aquatic humic material chlorination. The presence of these compounds (as their ethyl esters) will be substantiated by the GC/MS examination of these fractions. It will be recalled that trichloroacetic acid has already been established as a reaction product.

VII. OXIDATION OF AQUATIC HUMICS WITH PERMANGANATE

Aquatic humic matter (1.0 g) was methylated with diazomethane in the usual manner and subsequently reacted with excess $KMnO_4$ at 55°C for 6 hours. Untreated permanganate was destroyed with methanol, the mixture filtered, treated with cation exchange resin to bring down the pH to 2.9 and strip out associated Mn salts. The eluate was then concentrated on a rotary evaporator and further acidified with HCl to a pH 1.98. Extraction with ethyl acetate yielded 580 mg of product, a portion of which was treated with diazomethane in the usual manner to provide the methyl derivatives for GC/MS analysis.

In this case an excellent match of the EI and CI total ion chromatograms was achieved. This fact means that full use can be made of the two techniques in assigning structures to the components of this mixture. The total ion chromatograms are displayed in alternating fashion in Figures 2-7.

A. The first peak to be successfully identified was <u>dimethyl oxalate</u>. The library search resulted in a good fit with all indices above 900. The electron impact spectrum showed only a small molecular ion at m/e 118, a base peak at m/e 59 resulting from splitting the molecule in half and the protonated carboxyl group at m/e 45. The chemical ionization fragmentation pattern which is shown in Figure 8 confirms the assignment as outlined below. Both processes shown are common for esters.

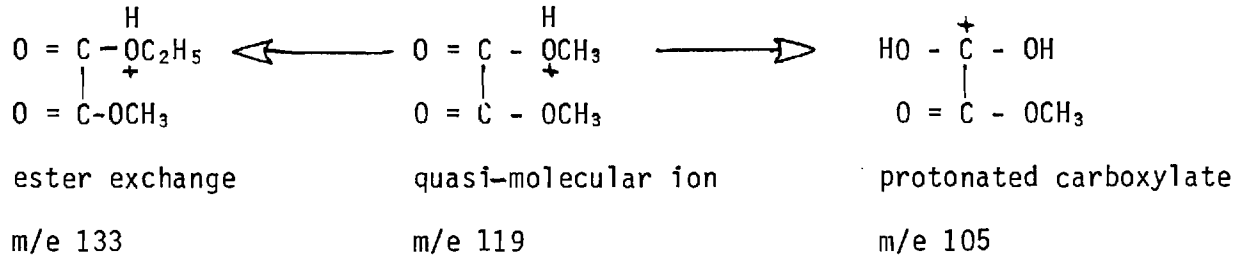
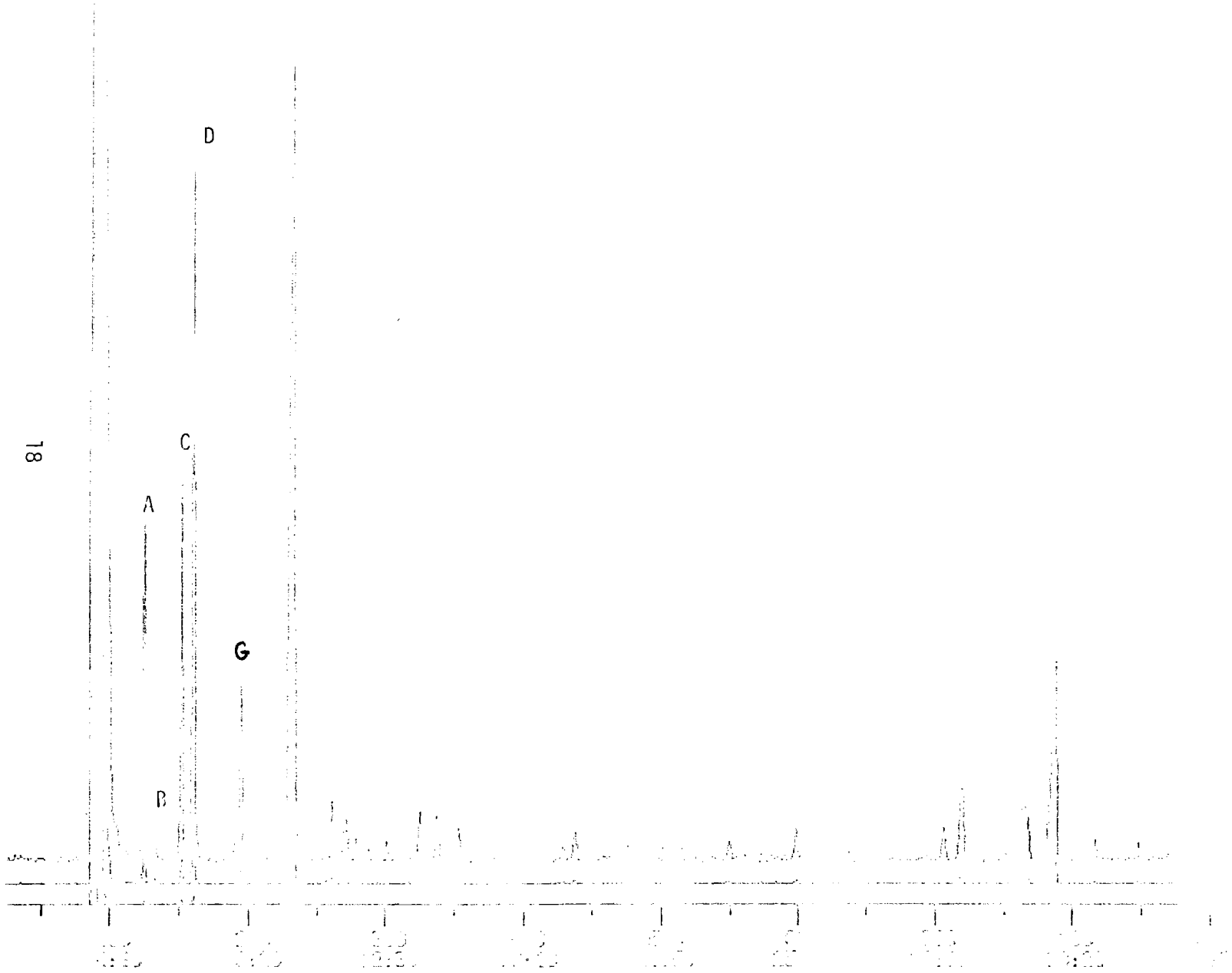


Figure 2. Mild Permanganate Oxidation of Humics.
CI Total Ion Chromatogram. Scans 0 - 1000.



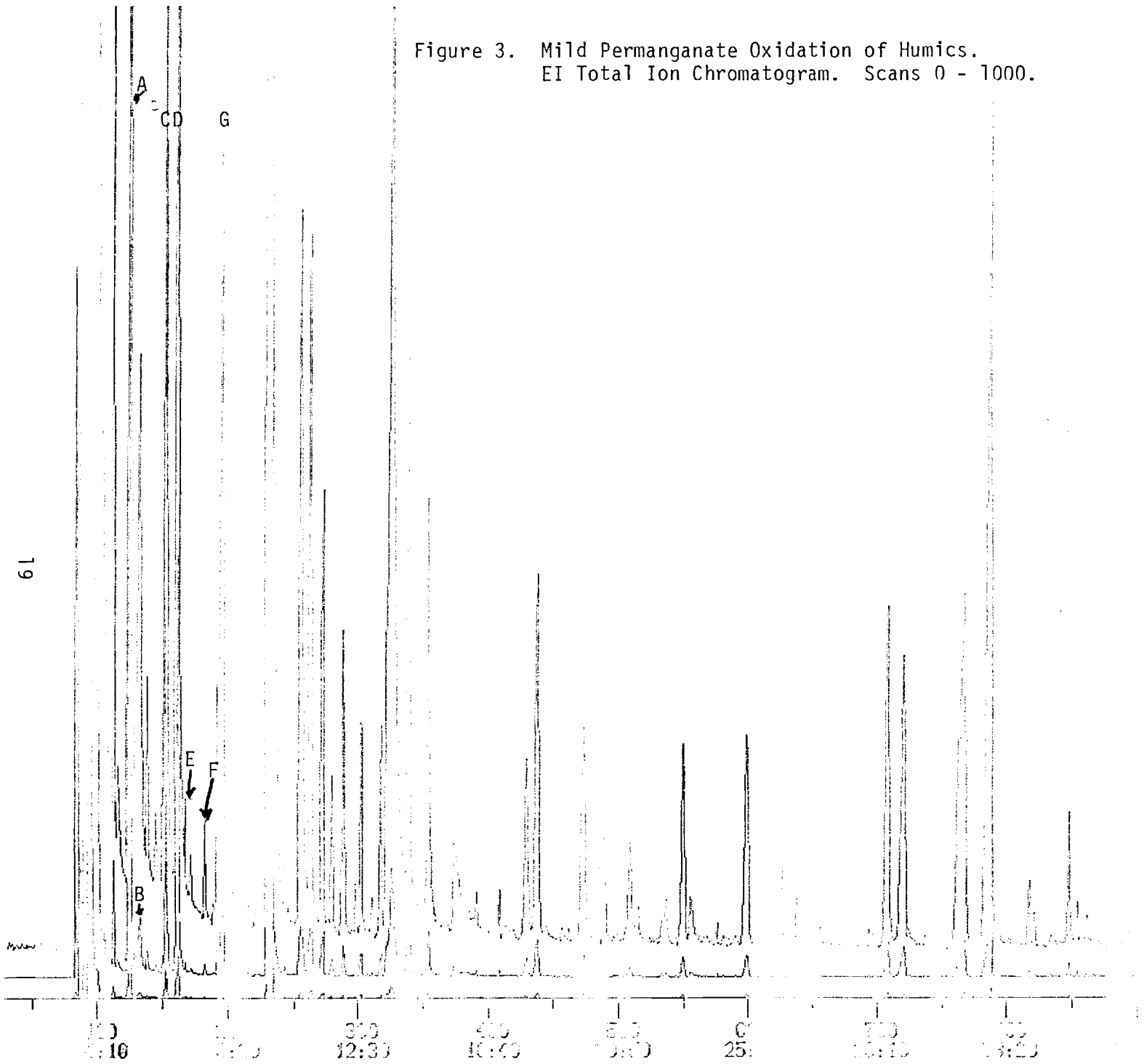


Figure 3. Mild Permanganate Oxidation of Humics.
EI Total Ion Chromatogram. Scans 0 - 1000.

Figure 4. Mild Permanganate Oxidation of Humics
CI Total Ion Chromatogram
Scans 1000-2000
(Note Scale is 10X greater than Figure 2)

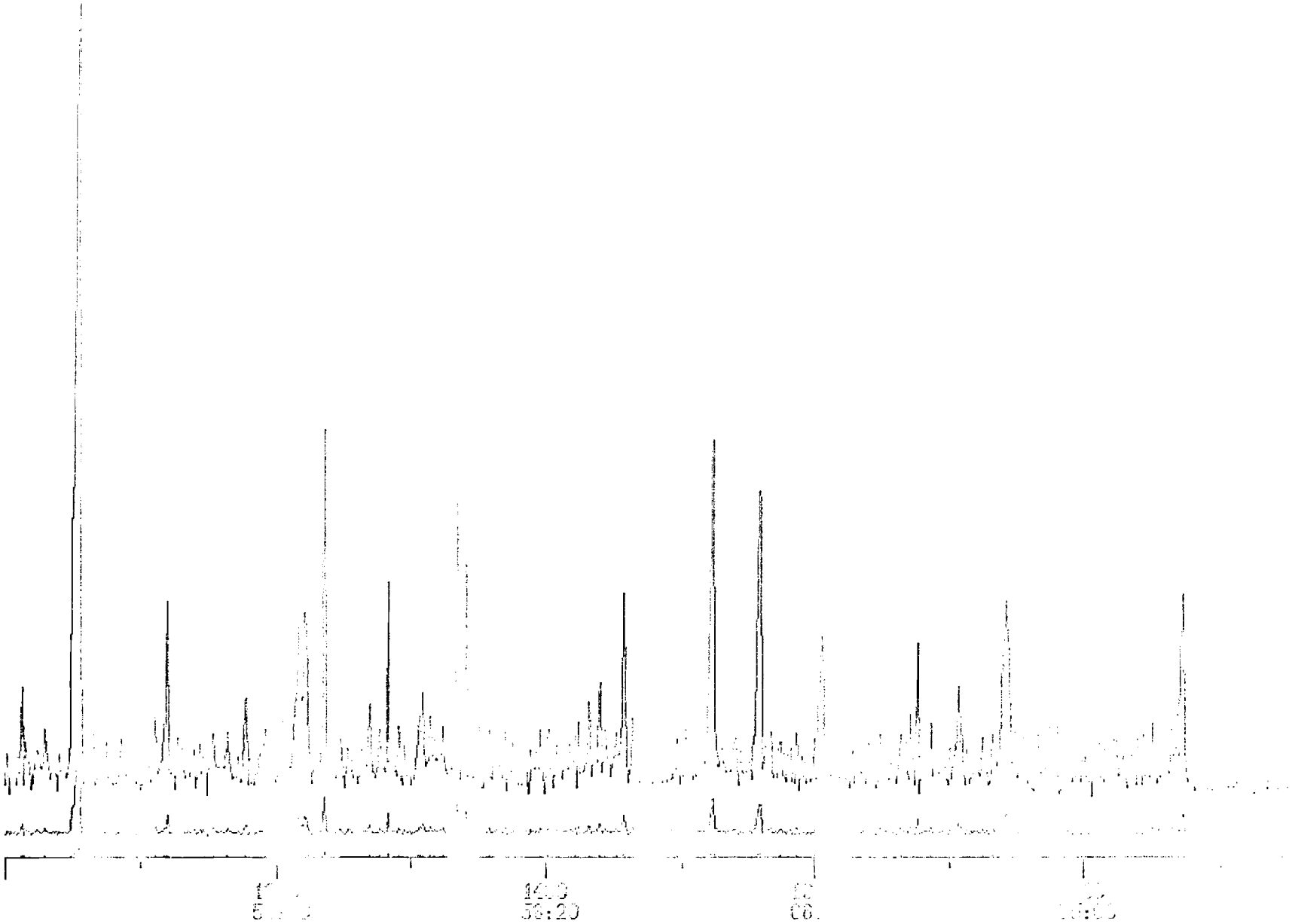
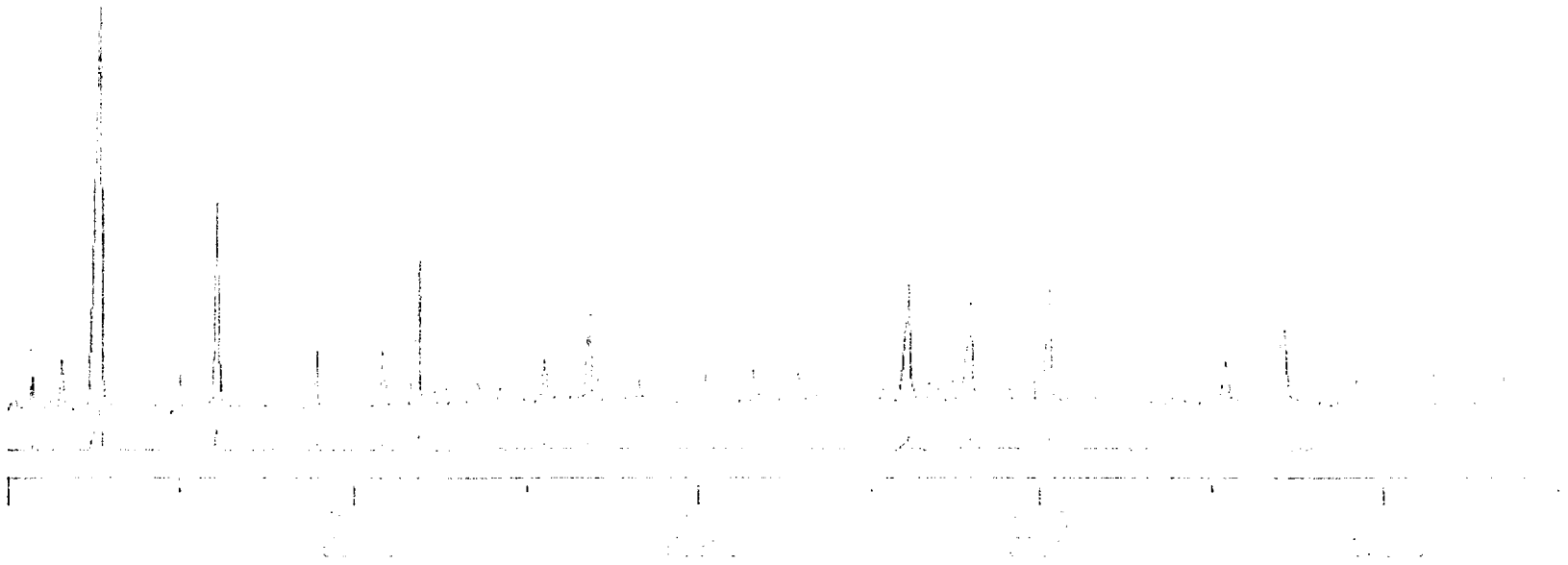


Figure 5. Mild Permanganate Oxidation of Humics
EI Total Ion Chromatogram
Scans 1000-2000
(Scale Same As Figure 3)



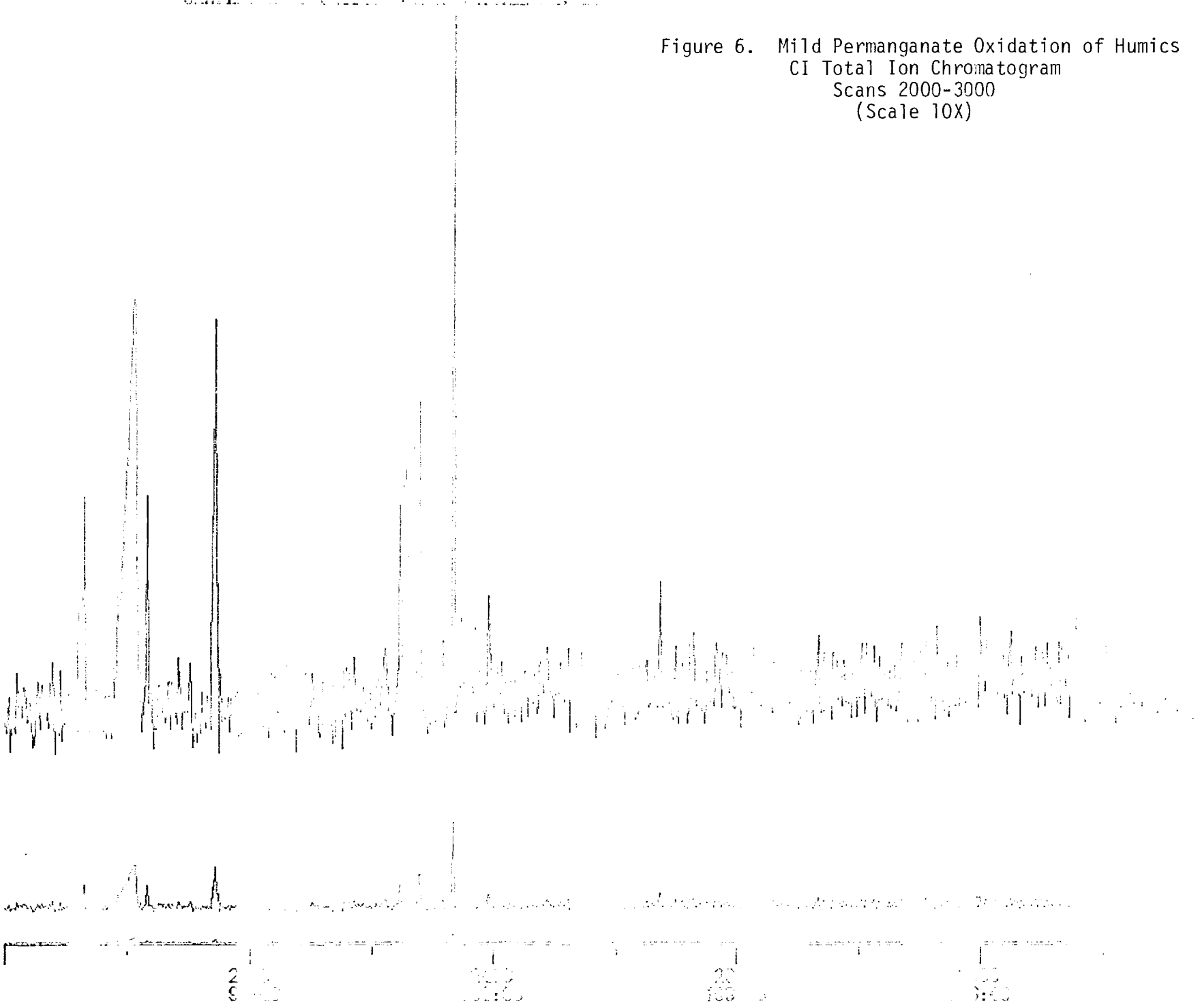


Figure 6. Mild Permanganate Oxidation of Humics
CI Total Ion Chromatogram
Scans 2000-3000
(Scale 10X)

Figure 7. Mild Permanganate Oxidation of Humics
EI Total Ion Chromatogram
Scans 2000-2550
(Scale 10X)

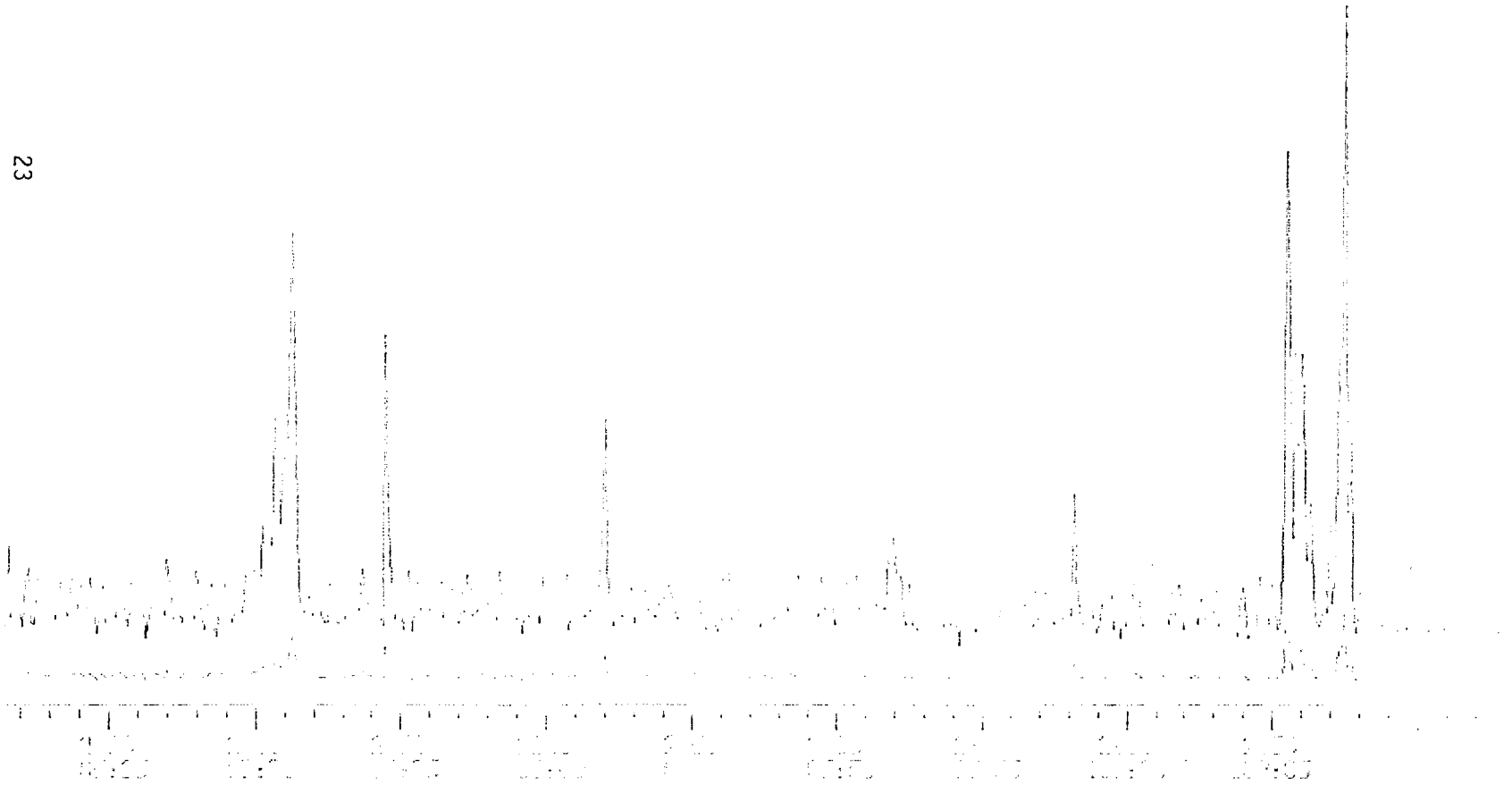
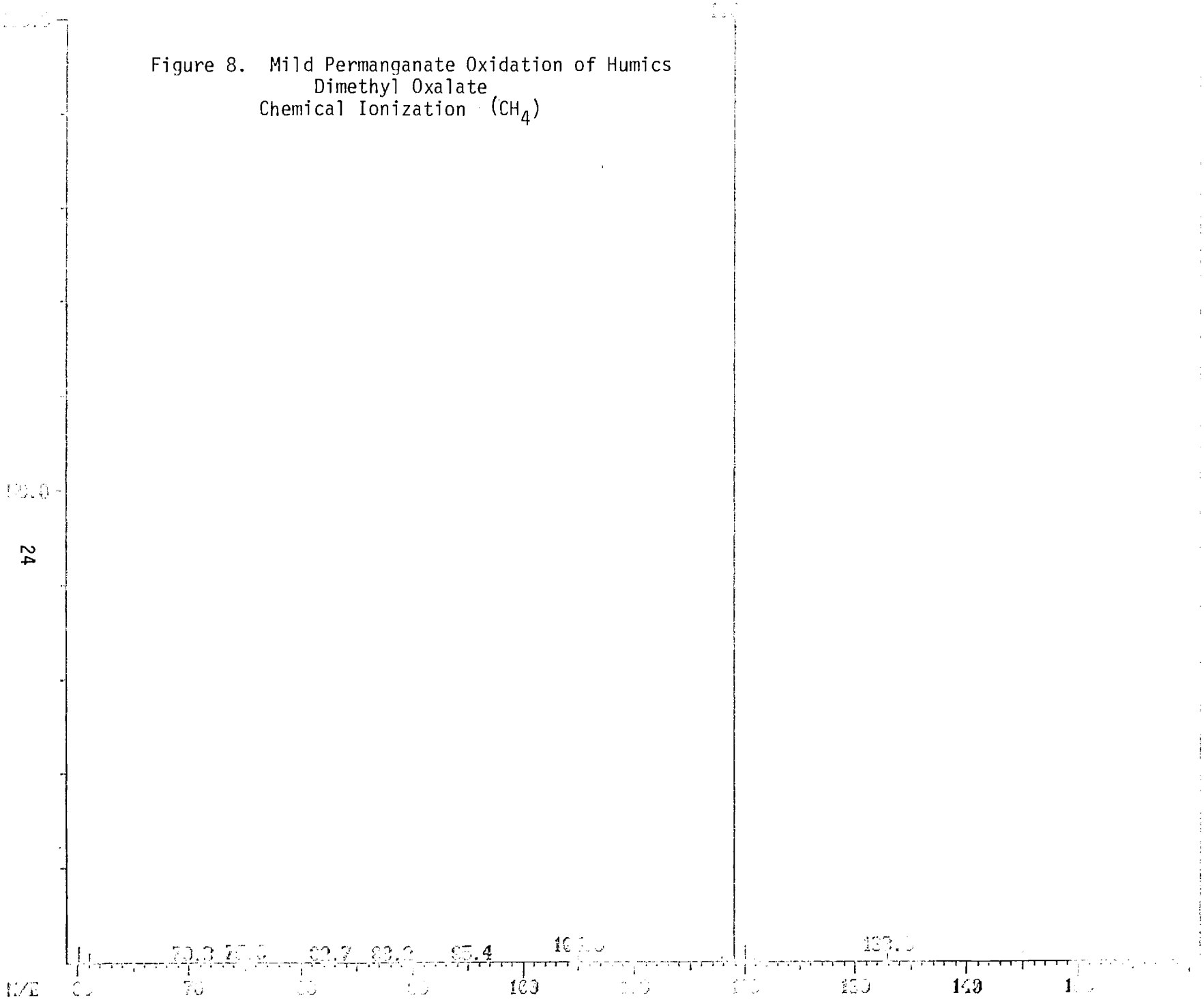
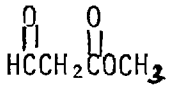


Figure 8. Mild Permanganate Oxidation of Humics
Dimethyl Oxalate
Chemical Ionization (CH_4)



B. This peak remains unidentified. While a tentative assignment of



METHYL 3-OXOPROPIONATE

explains the entire electron impact spectrum with the possible exception of m/e 57 and m/e 55, it is unreasonable to expect an aldehyde to survive the conditions of the reaction. The EI spectrum supports a molecular weight of the 102 with a loss of water from the quasi-molecular ion to form the base peak at m/e 85. Unfortunately the whole EI spectrum is weak and threrefore not of much help in aiding the structural assignment.

D. The dimethyl ester of malonic acid was the next peak to be positively identified. The EI fragmentation was characterized by a weak molecular ion at m/e 132, a strong loss of OCH_3 at 101 followed by loss of methanol to provide m/e 69, a $CH_3O - C = \overset{+}{O}CH_3$ ion at m/e 74, a methyl carboxylate at m/e 59 (base peak), a protonated carboxylate at m/e 45 and ketene at m/e 42. The chemical ionization fragmentation is supportive with M + 1 at m/e 133. The CI base peak at m/e 101 is derived from M + 1 by loss of methanol.

F. Methyl dimethyl malonate was tentatively identified on the basis of its electron impact spectrum which is shown in Figure 9. In this case, the molecular ion was not seen at m/e 146. A loss of OCH_3 which is a favored process in methyl esters was noted at m/e 115. Splitting the molecule at a accounts for the base peak at m/e 59 and the ion at m/e 87. A partial sequence is presented below. Unfortunately, the CI intensity was not high enough to provide a useful spectrum.

PLE: D/77 OXIDIZED HUMIC MATERIAL, EI

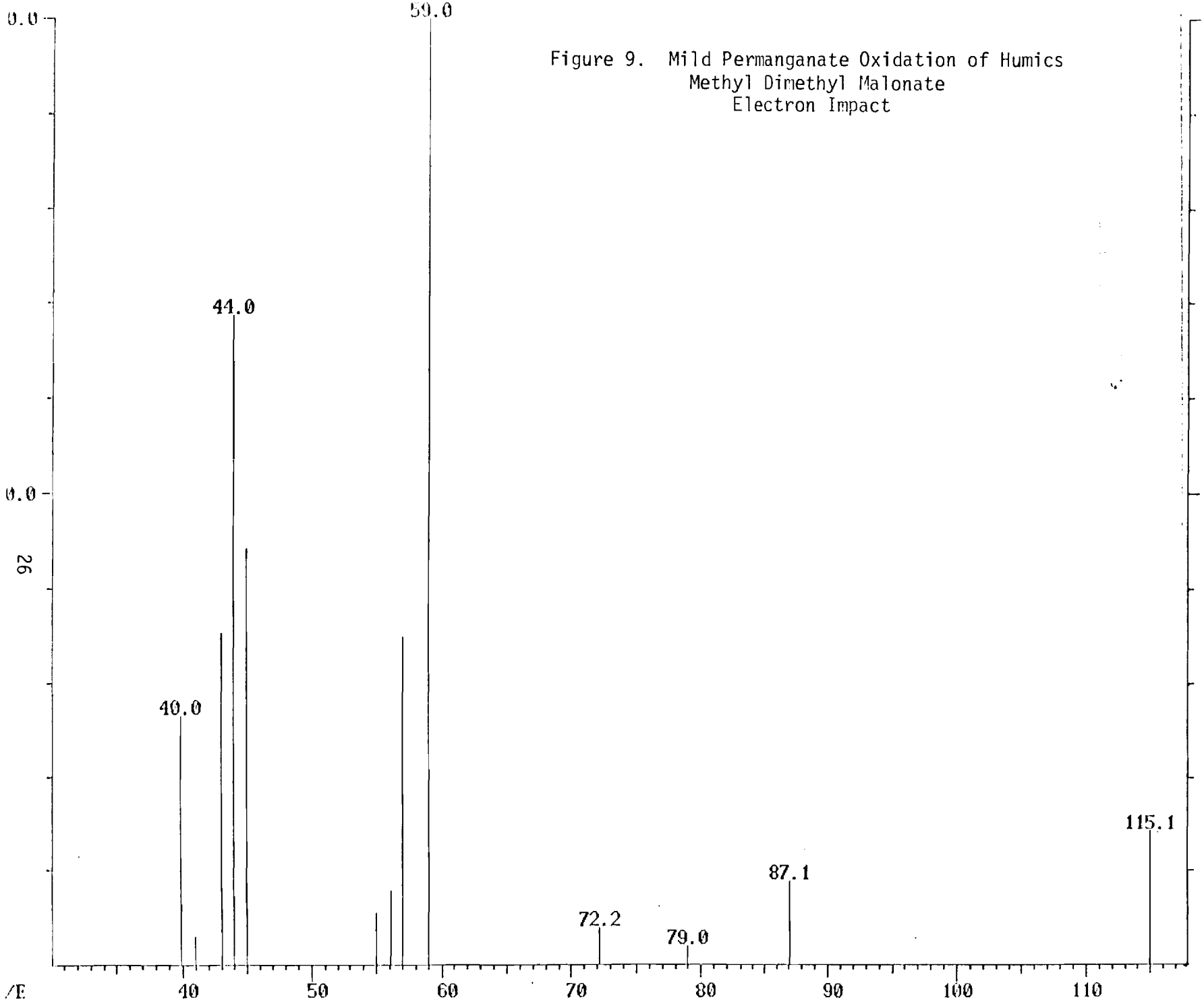
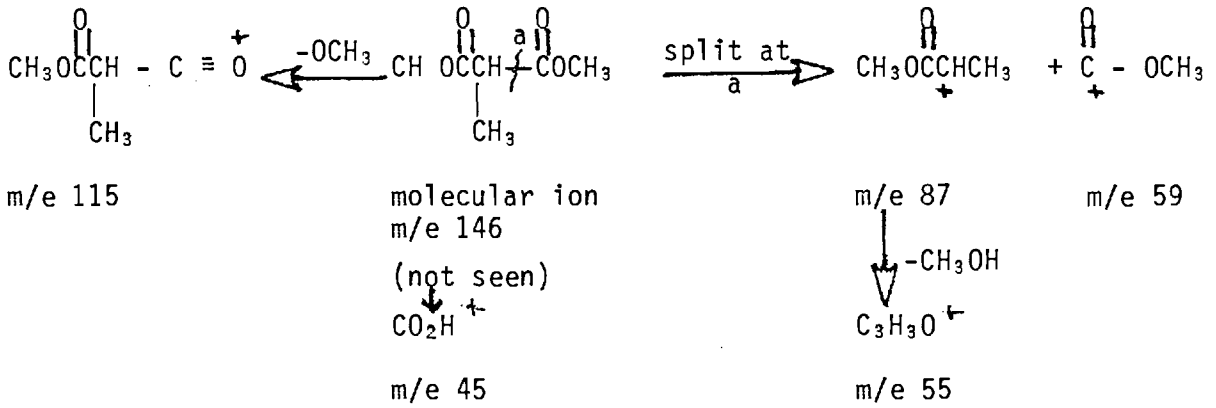
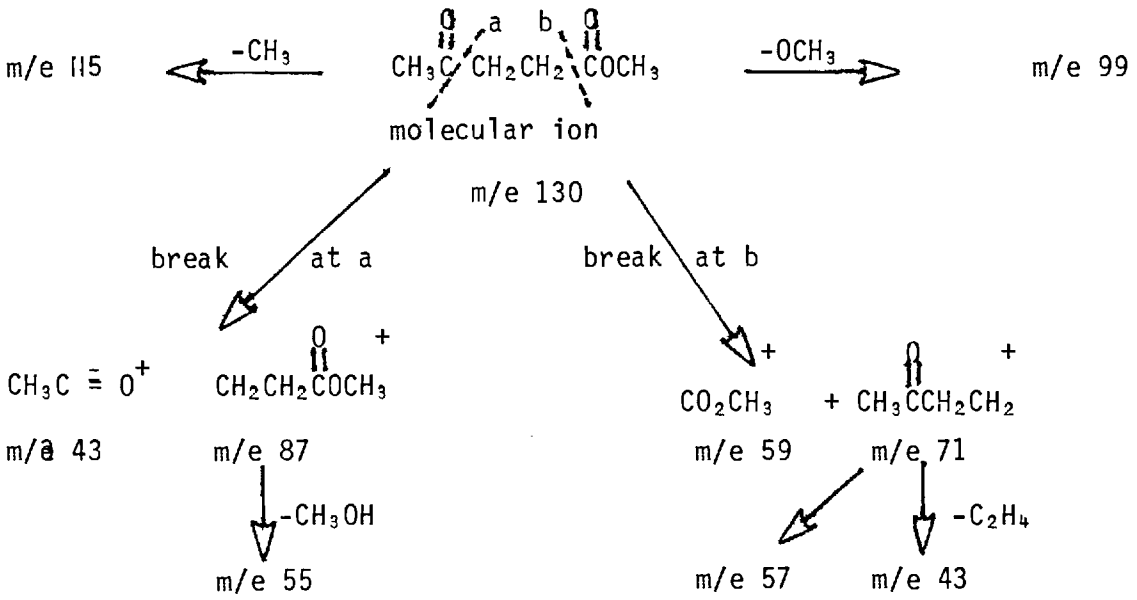


Figure 9. Mild Permanganate Oxidation of Humics
Methyl Dimethyl Malonate
Electron Impact



G. 4-Oxo-methyl pentanoate or methyl levulinate was the next organic acid identified by the GC/MS data system. In this case, the electron impact spectrum which is presented in Figure 10 exhibited a weak molecular ion at m/e 130. A description of the fragmentation is outlined below.



The ion at m/e 88 may represent a loss of ketene from M^+ but we cannot offer a mechanism at this time. The ion at m/e 45 is probably a protonated carboxyl group. The CI spectrum shows only the loss of methanol from the quasi-molecular ion at m/e 99.

SAMPLE: H/77 OXIDIZED HUMIC MATERIAL, EI

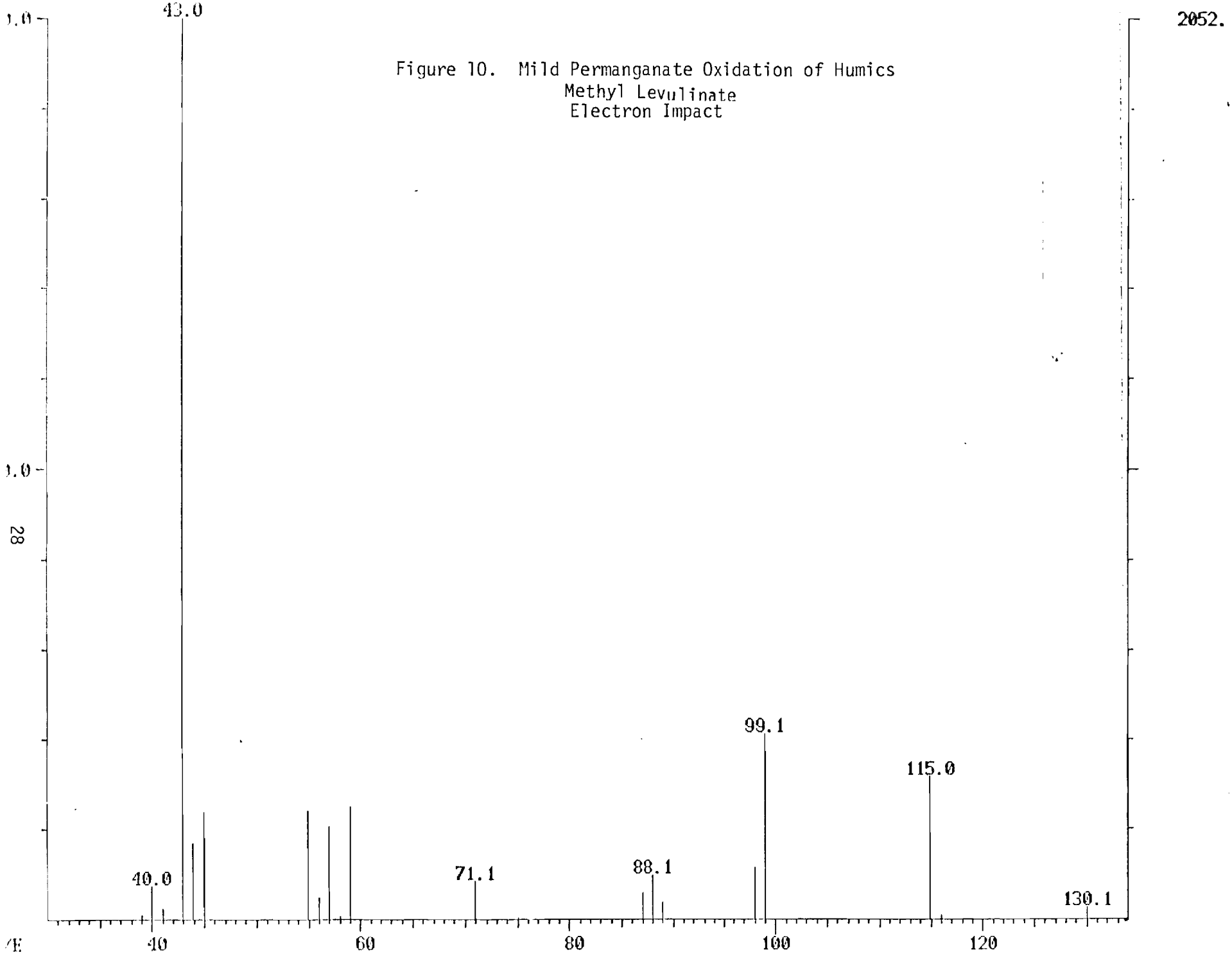


Figure 10. Mild Permanganate Oxidation of Humics
Methyl Levulinate
Electron Impact

VIII. APPLICATION OF AN ON-COLUMN EC DETECTOR

A miniaturized, porous anode EC-type detector, the design of which was conceived by Mr. J. D. Lupton has been successfully mounted on-column and is currently being tested with standards. Although this work is in a very early stage, detector performance using packed columns appears to be acceptable. Application of this fundamental advance, which was made independently of project support, should have significant utility in connection with the successful execution of this work. Further details will be presented in future reports.

IX. ISOLATION OF AQUATIC HUMICS

The third batch of water from Satilla River (about 110 gallons) is being processed for the isolation of aquatic humics. As noted elsewhere in this report, a subsample of this raw water is being processed for the separation of other classes of naturally occurring organic material. In addition, the raw water has also been subjected to simulated disinfection with chlorine using the mini-pilot facility.

X. BROMINATION OF AQUATIC HUMICS

A sample (M/30, 100 mg) of aquatic humic material was dissolved in water (20 ml) and treated with excess bromine (1 ml). It was shaken occasionally and left overnight. Bromine was driven off in a slow stream of nitrogen, and the aqueous solution extracted with ethyl acetate for 3 hours. The extract was dried and the solvent removed leaving behind a residue which was then methylated with diazomethane. The product is partially soluble in either $CHCl_3$ or ethyl acetate. The solution shows several peaks on a 3% OV 17(6'x1/8")

column. A more comprehensive GC/MS analysis will be carried out during the next reporting period.

XI. ESTABLISHING A TEST PROCEDURE FOR OZONE

In the course of renewing our efforts to compare the chemical activity of ozone from several types of generators, it was discovered that the use of the $Mn^{+2} \longrightarrow Mn^{+7}$ reaction as an indicator was not always reproducible. Accordingly, we have begun a series of tests varying the pH, the temperature, the Mn concentration and the time of ozone exposure. The glassware is being rigorously cleaned to prevent MnO_2 deposits from interfering with the reaction. Reproducibility is already much improved on the basis of preliminary experiments which documented the importance of acid and Mn^{+2} concentration.

XII. ESTIMATIONS OF CARBOXYL CONTENT

The studies recently described in past progress reports have been supplemented by thermometric titration of a sample (M/30) of aquatic humics done by Edward M. Perdue at Portland State University. These studies reveal that sodium hydroxide neutralizes 4.4 meq/g of -COOH groups and 1.2 meq/g of phenolic hydroxyls when titrated with aquatic humics. An abstract of these findings, together with our data have been communicated to the Geological Society of America for presentation at its forthcoming annual meeting in Toronto. A copy of the abstract is attached at the end of this report.

The distribution of acidic functional groups in our Satilla River aquatic humics had been analyzed in previous years (Martin, 1973). The same total acidity was found ($\sim$ 10.4 meq/g). The carboxyl group content was analyzed by the Ca-acetate method and evaluated at pH 9.8 ($\sim$ 6.2 meq/g), giving a phenolic

hydroxyl content (by difference, i.e., total acidity minus carboxyl groups, in meq/g) of 4.2 meq/g. So the ratio of carboxyl/phenolic OH was believed to be $\sim$ 1.5. Our new critical evaluation of the Ca-acetate method (i.e., lack of specificity for COOH), corroborated by Dr. Perdue's calorimetric data and our acid-base titrations, makes it evident that the correct ratio (for sample material collected from the same site) is between 0.7 and 0.8. From this finding it appears that we have to revise our ideas about the close chemical relationship between our river water humics and soil fulvic acids.

XIII. REACTION OF AQUATIC HUMIC MATERIAL WITH CHLORINE

As of this writing, a total of five separate runs with aquatic humics have been carried out using the mini-pilot facility. The products from all of these reactions, sampled from the settling chamber, the sand filter effluent and the influent reservoir (control) have been worked up and analyzed by GC/MS. The conditions employed for each of the reactions is outlined in Table III.

Some general conclusions based on the relative abundance of chloro-organics in the ethylated product mixtures can be made in spite of the fact that all of the acquired data have not yet been thoroughly evaluated. These findings are described in the following paragraph.

The chlorine concentration appears quite critical. In very similar runs at the same pH (7.3), the run (II-110) with 1630 mg of chlorine provided an abundance of chlorinated products while the run with 1145 mg gave only a few chlorinated compounds.

The run (II-134) at low pH (6.0) and low chlorine concentration (575 mg) did not seem to produce any chlorinated compounds even though the contact times were longer than in other runs.

Table III. Summary of Reaction Conditions.

Run	II-110	II-18	II-134	II-145	II-149
Buffer Component #1	10g K_2HPO_4	10g K_2HPO_4	17g KH_2PO_4	1g $NaHCO_3$	0.15g $CaCl_2$
Buffer Component #2	3g KH_2PO_4	3g KH_2PO_4	3g K_2HPO_4		5g $NaHCO_3$
Flocculant	None	None	1g $Al_2(SO_4)_3$	860mg $Al_2(SO_4)_3$	860mg $Al_2(SO_4)$
Aquatic Humic Matter	100 mg	100 mg	100 mg	100 mg	100 mg
Volume of Water	10 liters	10 liters	10 liters	10 liters	10 liters
pH	7.29	7.26	6.03	7.90	8.19
Chlorine Added to 8.6 l of Solution	1636 mg	1145 mg	575 mg	756 mg	850 mg
Time Sand Filter Effluent Quenched	8 hrs	8 hrs	M*	8 hrs	9 hrs
Time Settling Chamber Quenched	20 hrs	8 hrs	M	8 hrs	9 hrs
Simulated Reservoir Quench	M	8 hrs	M	8 hrs	9 hrs

* M = missing information

Runs at pH 7.9 and 8.2 gave abundant chorinated compounds even with lower levels of chbrine - i.e., 756 mg and 850 mg respectively.

The formation of solvent extractable chlorinated compounds from aquatic humics thus appears to be related to pH and chlorine concentration; higher pH and higher chlorine concentration seem to favor the formation of these materials. This does not necessarily mean that the chlorination reaction proceeds more rapidly at higher pH. It may be that the breakdown of chlorinated products into small molecules which can be found by GC/MS proceeds more effectively at high pH or it may mean that a higher pH speeds the hydrolysis of aquatic humic matter so that precursor concentrations rise faster than reaction rates can decrease . . It is evident that a great deal of additional work will have to be done in order to clarify these points. A partial summary of the GC/MS results is presented in the next section.

XIV. ANALYSIS OF PRODUCTS - CHLORINATION OF AQUATIC HUMICS

In view of the fact that the amount of data acquired to date is multiplying at a truly amazing pace, considerable effort has been devoted towards developing an organized approach to interpreting and presenting the data. Increased quality control will assure that instrument response to decafluoro triphenyl phosphine meets EPA specifications prior to every run. A retention time specification will also be instituted so that run-to-run comparisons can be more readily performed. In the meanwhile, Table IV represents a summary of the interpreted data to date. The lettering of peaks in the total ion chromatogram is based upon run II-110, sand filter eluate. As new peaks are discovered prior to A, they are designated PRE A-1, PRE A-2, etc. New peaks identified between A and B are labled A-1-B, A-2-B, etc. Major peaks not identified will, nevertheless, be assigned letters according to the

Table IV

Chlorinated Aquatic Humics Product List

		Run No.		
		II-110 Sand Filter	II-134 Sand Filter	II-124 Reservoir Control
Pre A-1	3-Methyl-2-butanone	ND*	X***	X
A	3-Chloro-2-methyl-1-butene	X	X	ND
A-1-B	Heptane	ND	X	X
A-2-B	2-Chloro-3-methyl-2-butene	ND	X	ND
B	2-Methyl-3-pentanone	X	X	X
B-2-C	3-Methyl-2-butanol	ND	X	ND
C	3-Chloro-2-methyl-2-butanol	X	-**	ND
D	4-Hydroxy-3-methylbutanal	X	-	X
E	2,3-Dichloro-2-methylbutane	X	-	ND
F	1-Chloro-2-methyl-2-butanol	X	-	ND
G	1,3-Dichloro-2-methylbutane	X	-	ND
H	3-Chloro-2-methyl-1-butanol	X	-	ND
I	$C_5H_{11}ClO$	X	-	ND
J	N-Nitrosodiethylamine (artifact)	X	-	X
K	$C_5H_8Cl_2$	X	-	ND
L	4-Methyl-3-heptanone	X	-	X
M	$C_7H_{12}O_2$ (?)	X	-	X
N	1,4-Dichloro-2-methyl-2-butanol	X	-	ND
O	Ethyl Trichloroacetate	X	-	ND

*ND indicates looked for but not detected.

**- indicates analysis incomplete.

***indicates detected.

above rules. This same general plan will be followed in organizing and presenting the data from other reaction sequences. Thus a section may begin with a subheading such as D and skip letters in order that the reviewer may proceed from one report to another with a minimum of confusion.

Thus it can be seen that every non-halogenated compound isolated from the reaction mixture is also present in the control. No halogenated compounds have been found to date in the control. Many compounds present in both the control and the reaction mixtures await identification. It is our intention to present an expanded version of this table in subsequent reports. Considerations of time and space do not permit us to outline the basis for the assignment of new structures or to fully discuss the analysis of the reaction mixtures and controls. This information is being assembled in logical order and will be presented in future reports.

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- extraterrestrial
- general
- historical
- history of
- marine

- mathematical
- Precambrian
- Quaternary
- structural

geomorphology

geophysics

geoscience information
hydrogeology
mineralogy/crystallography
paleomagnetism
paleontology/paleobotany
- invertebrate
- micro
- paleobotany
- palynology
- vertebrate

petrology
- experimental
- igneous
- metamorphic

sedimentology
- sedimentary petrology

stratigraphy
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OTHER
Organic Geochemistry

ACID-BASE PROPERTIES OF AQUATIC HUMIC SUBSTANCES

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Recent investigations of the nature and quantities of acidic functional groups of aquatic humic substances have revealed a close chemical relationship of the latter to soil humic substances. However, significant discrepancies appear upon comparison of different titrimetric methods. Carboxyl group content of aquatic humics extracted from the Satilla River (Georgia) determined by titration with base in 0.1 N NaCl (5.0 meq/g) is consistently lower than that after reaction with Ca-acetate, especially when the latter is evaluated at pH 9.8 (6.8 meq/g) rather than at the equivalence point (5.6 meq/g). Removal of colored humics after Ca-acetate reaction but prior to titration by ultrafiltration only decreases this difference (5.2 meq/g) but does not eliminate it. Standard filtration techniques have no effect.

Thermometric titrations of the same aquatic humic material yield a curve whose linear region has a slope of -13.6 kcal/mole characteristic of carboxyl groups, while the nonlinear region resembles the titration curves of phenolic hydroxyl groups. A standard graphical extrapolation of initial and final slopes in the titration curve shows that the aquatic humics contain 4.4 meq/g carboxyl and 1.2 meq/g phenolic hydroxyl groups.

By comparison of the combined amount of 5.6 meq/g titratable acidic functions with that found in the Ca-acetate reaction, it is suggested that Ca-acetate raises the acidity of certain phenolic hydroxyls so that they become titratable down to pH 7.8. From the total acidity (11.0 meq/g) the amount of phenolic hydroxyl is estimated at 6.0 - 6.6 meq/g. Thus, only a relatively small portion of phenolic hydroxyl (18-20%) can be titrated with sodium hydroxide under our experimental conditions.

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